Resource partitioning between the cryptic species Brandt's bat (*Myotis brandtii*) and the whiskered bat (*M. mystacinus*) in the UK

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ABSTRACT

Similarities in echolocation and morphology suggest that the ecological niches of the cryptic species *M. mystacinus* and *M. brandtii* overlap. However, in order for animal species to coexist sympatrically, it is expected that they will occupy different ecological niches. Because the bats have different evolutionary histories, I therefore wanted to investigate how species which are morphologically similar, but not closely related, partition their resources.

There is currently great confusion in how to distinguish between *M. mystacinus* and *M. brandtii*; establishment of easy and reliable identification features based on gene sequencing is therefore critical. All morphological features tested showed some overlap between the two species. However, when used in combination; penis shape, upper jaw dentition, lower jaw dentition, tragus shape and thumb claw length could distinguish between the species with 100% confidence.

There were several differences in the bats' foraging ecologies. *M. mystacinus* used grassland over all other habitat types, whereas *M. brandtii* used coniferous woodland. The habitat use results in addition to other results in this study; therefore indicate that coniferous woodland may be more important as foraging habitat for bats than previously assumed.

M. mystacinus emerged earlier, changed day roosts less frequently, had longer flying times and also showed greater foraging site fidelity and less foraging site overlap than *M. brandtii.* There were also significant differences between their diets, but both species have a broad diet comprised mostly of Diptera and Lepidoptera with a proportion of their prey being gleaned. The bats show seasonal differences in their dietary diversity and composition.

The results suggest that ecological differences can occur between bat species that are virtually identical in morphology, but not closely related. Morphological differences may therefore be a weak indication of any ecological differences between species. This needs to be taken into account when managing these and other cryptic bat species for conservation.

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AUTHOR'S DECLARATION

This thesis and the data presented in it are the results of my own original work, except where due acknowledgement or reference has been made. No part of this thesis has previously been submitted in any previous application for a higher degree at this, or any other, university. The views expressed in this thesis are my own and not those of the university.

Lene Berge

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COLOUR CODES FOR HABITAT MAPS

Grassland

Arable land

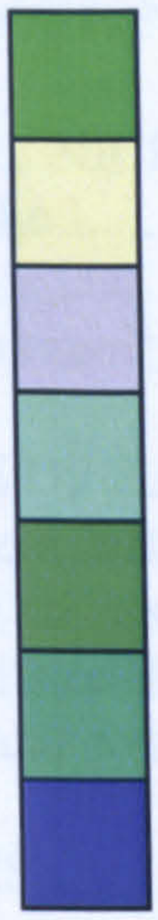
Built up areas

Deciduous woodland

Coniferous woodland

Mixed woodland

Water



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1.1. THE ECOLOGICAL NICHE AND RESOURCE PARTITIONING

1.1.1. The ecological niche

To conserve an animal species effectively, we need information about all aspects of its biology such as birth, death and migration rates, what resources and conditions it requires, in addition to understanding the inter- and intra-specific interactions that it experiences (Begon et al. 1990). Such interactions include predation, parasitism, mutualism and competition. This information was first summarised and formally described by Hutchinson (1957) as the ecological niche. The ecological niche may be defined as the way in which an organism interacts with its environment. It is important to keep in mind however, that the niche only applies to a single instant in time. The fundamental niche is the niche an organism would occupy if competitors were not present. The realised niche is the niche it occupies under natural circumstances, where it maintains a viable population in the presence of competitors and predators. Knowing a particular species' ecological niche would therefore allow the identification of all the habitats where it could exist. The ecological niche is shaped by biotic and abiotic factors. Abiotic factors refer to the non living parts of the ecosystem such as rainfall, temperature and soil conditions, while biotic factors refer to the living parts of the ecosystem such as vegetation, decomposers, herbivores and carnivores. Fundamental features of the niche include differences in diet, habitat use and microhabitat use (see Chapter 3 and 5).

1.1.2. Competition

Competition is defined as an interaction between individuals of the same species (intra-specific competition) or different species (inter-specific competition). It is brought about by a shared resource in limited supply and leads to a reduction in the survivorship, growth and reproduction of the competing individuals (Begon et al. 1990). Competition can also occur when the resource is not in short supply, but the two species interfere with each others use of the particular resource. If the niches of sympatric species do not differ sufficiently, competition will occur. More than 70 years ago, Gause (1934, 1935) formulated what is referred to as the principle of competitive exclusion, based on a laboratory study on competition among three species of *Paramecium*. The principle states that if two species are competing with each other for the same limited resource, one of the species will be able to use that resource more efficiently and eventually out-compete the other. Another outcome of competition among sympatric species is natural selection making the species evolve to

become more different (Wiens 1989) because two species cannot occupy the same niche indefinitely (Raven and Johnson 1996). Examples from nature include MacArthur's study on competition where he demonstrated that although five species of New World warblers (Parulidae) feed on insects in the same spruce tree simultaneously, they show temporal and physical division by feeding in different parts of the tree and in different ways (MacArthur 1958).

1.1.3. The biological community

The biological community is defined as the species that occupy a particular locality and includes the interactions among those species (Primack 1998). The composition of communities is affected by competition and predation (Ricklefs 1990, Terborgh 1992, Gotelli 1995). Competing species reduce the competition by resource partitioning; animal communities can therefore be described in terms of the resource partitioning between its members (Ricklefs 1990). It is important to keep in mind however, that a range of other factors such as chance, dispersal abilities, habitat change, speciation and extinction often influence the evolutionary histories of animal communities (Wiens 1989). A common means by which closely related species are able to coexist is the evolution of morphological differences that enable them to utilise different resources or slightly different types of habitats (Kimmins 1997). Two well-known examples are the Darwin's finches (Fringillidae) of the Galapagos (Lack 1947) and the honeycreepers (Drepanididae) of Hawaii (Takakazu 2005). Beak length varies significantly among each of these two groups of closely related bird species. The different beak sizes restrict feeding to different types of food, thereby allowing the birds to reduce inter-specific competition.

1.2. THE STRUCTURE OF BAT COMMUNITIES

1.2.1. The order Chiroptera (bats)

The order Chiroptera (bats) comprises over 1000 species worldwide and is the second most species-rich mammalian order (Simmons 2005). Bats are found on all continents, except Antarctica, but are most abundant and diverse in the tropics (Findley 1993). They eat a variety of foods and may be insectivorous, carnivorous, nectarivorous, frugivorous, piscivorous, sanguinivorous or omnivorous. However, all European bat species are insectivorous (Hill and Smith 1984).

Chiroptera have traditionally been divided into the suborders Megachiroptera and Microchiroptera. Megachiroptera comprised the old-world flying foxes (Pteropodidae), while Microchiroptera comprised just over 800 species in 17 families (Corbet and Hill 1991). Unlike the megachiropterans, all microchiropteran bats used true (laryngeal) echolocation. All microchiropterans were of small size (2- 200 g) and unusually for a small mammal they were K-strategists; being long-lived (up to 30 years), slow reproducing (commonly one young per year, more rarely twins), had high adult survivorship (50- 80% per year) and maintain relatively stable populations (Hill and Smith 1984). Recently, molecular studies have revealed that these two groups are artificial. Molecular phylogenetic data suggest that the families Rhinolophidae, Hipposideridae, Craseonycteridae, Rhinopomatidae and Megadermatidae belong to the suborder Yinpterochiroptera along with megabats (family Pteropodidae), while the family Nycteridae belongs to the suborder Yangochiroptera along with vespertilionids, noctilionids, emballonuroids and nine other families (Teeling et al. 2002).

1.2.2. Wing morphology and its relation to bat ecology

Bats often differ in wing morphology and echolocation call structure, because these factors influence which habitats they exploit, which insects they are able to detect in a particular habitat and how far they can fly to reach them (Norberg and Rayner 1987, Neuweiler 1989, Fenton 1990). Wing morphology can most easily be understood in terms of wing loading, aspect ratio and shape of the wing tips. Wing loading is described by body weight divided by wing area (N/m^2), which provides a measure of the size of the wings compared to the body mass of the bat. Wing loading is positively correlated with minimum speed and negatively correlated with manoeuvrability and agility. Aspect ratio, described by square of wingspan divided by wing area, refers to the shape of the wings. A high aspect ratio, referring to long, thin, wings, corresponds with relatively low drag and hence aerodynamic efficiency in flight. The wing tip index is a measure of the shape of the wing tips. Pointed wing tips are negatively correlated with manoeuvrability. Manoeuvrability is favoured by low wing loading, aspect ratio and wing tip index (Norberg and Rayner 1987). Note that bats, which by their wing morphology are predicted to fly in clutter, are able to enter open spaces. On the other hand, bats adapted to fly in open areas may not be able to forage in clutter (Brigham et al. 1992).

1.2.3. Echolocation and its relation to bat ecology

The type of echolocation call a bat uses will determine the amount of clutter in which it can detect obstacles, whether or not it can detect individual prey targets and separate them from

the background echo clutter and from what distance it can detect its prey (Simmons et al. 1979, Simmons and Stein 1980, Neuweiler 1989). Echolocation calls of high frequency attenuate more quickly than those of lower frequency (Lawrence and Simmons 1982) and are therefore unsuitable for long-range echolocation needed in open spaces (Dusenbery 1992). High frequency calls are more effective in woodland because clutter echoes (echoes from objects other than the target of interest) are reduced. Bats that operate at low duty cycles, i.e. with their echolocation signal on for <30% of the time (Jones 1999) use short call durations when flying close to obstacles so that echoes of interest return after call emission has finished and auditory sensitivity is improved. Consequently, manoeuverable flight in cluttered environments requires low-intensity, short calls (or constant frequency (CF) echolocation with Doppler shift compensation (DSC)) to avoid pulse overlap and to detect obstacles more easily. Fast flight in open environments on the other hand, requires high-intensity, far-ranging echolocation calls (Neuweiler 1983, Fenton 1990). Flight and echolocation are adaptively linked and as a result, bat species with similar wing morphologies tend to produce similar types of echolocation calls (Aldridge and Rautenbach 1987). However, because behaviour is so flexible, individual animals may behave in a way that a study of their morphology and echolocation would not predict.

1.2.4. Bat communities and competition

One of the key questions in community ecology is whether communities are random assemblages of species structured by stochastic factors or whether structure occurs as a result of competition. Theoretically it is believed that if animal communities were structured by competition this would result in great morphological and ecological differences among taxa (Hutchinson 1957, MacArthur and Levins 1967). However, little evidence has been found for competition structuring bat communities (e.g. Fleming et al. 1972, Fenton 1982, Patterson et al. 2003). Bats appear to be adapted to stable communities with clearly defined, but overlapping ecological niches (e.g. Tamsitt 1967, McNab 1971, Fleming and Heithaus 1986, Findley 1993). It is believed that resources available to bats have been abundant in the evolutionary past of bat communities when there was little competition and tightly-packed communities evolved (Wiens 1989). Today, these communities are limited by resources shared by many species. However, recent studies suggest that stochastic factors are important in shaping bat communities and that competition plays a minor role (Findley 1993, Arita 1997). The relative roles these factors play have not yet been resolved (Ricklefs 1990). It appears that competition occur mainly between similar or related species, or within a

particular guild (Findley 1993). It is also important to note that competitive interactions not only cause morphological evolution or extinctions. If competition is not sufficiently intense, it may only alter abundances of local populations of interacting species (Patterson et al. 2003). If morphological similarity reflects ecological similarity, the species which are morphologically similar should experience the highest degree of competitive pressure and the lowest abundance. A simulation model showed no clear signs of density compensation of five bat ensembles in the New World. The model showed that gleaning carnivorous bats were most affected by interactions between nearest neighbours (Stevens and Willig 2000). Fenton (1982) concluded "A consideration of the available literature (on insectivorous bats) provides no convincing evidence that bats specialize by the timing of activity, diet, use of habitat, foraging strategy or morphology...There is still no clear picture of how sympatric insectivorous bats partition food resources, or if, indeed they do". After reviewing recent ecological studies on bat ecology however, Patterson et al. (2003) concluded that differential habitat use may be a principal avenue of resource partitioning by bats. Note however, that a large proportion of the studies of resource partitioning of bats that have been reported are of bats belonging to the genus *Myotis* (Arlettaz 1999). Arlettaz (1999) argues that this may be due to many *Myotis* bats being gleaners and this being a more predictable resource leads to competition.

1.3. CRYPTIC SPECIES

1.3.1. Morphology and competition

Understanding the biological mechanisms that allow species to exist in sympatry is one of the major challenges for community ecologists (Ricklefs 1990). Bat communities often comprise a majority of morphologically very similar bat species, with only a few outlying forms (Findley 1993). This observation has made many bat biologists reach the conclusion that resource partitioning may play a minor role, because resemblance in wing morphology and echolocation is assumed to reflect similarity of ecological niches (e.g. Aldridge and Rautenbach 1987, Crome and Richards 1988, Willig and Moulton 1989, Fenton 1990, Findley 1993, Arita 1997). Bat species with similar morphologies are assumed to be ecologically similar, thus making it difficult to explain their coexistence. The ecological niche theory suggests that two species will occupy different ecological niches when occurring in sympatry (Hutchinson 1957). This is because morphological features such as wing morphology and

echolocation call structure are believed to play a major role in determining where and how different bat species hunt, consequently, species which are morphologically similar are believed to show little niche separation. However, although large morphological differences appear to have a profound influence on the foraging ecology of bats, the influence of smaller differences is unknown, and the mechanisms that allow similar species to coexist have yet to be determined.

A molecular study by Thabah et al. (2006) on the Indian bat *Hipposideros larvatus sensu lato* showed that in fact two cryptic species are present. One species echolocating at 85 kHz had larger ears and longer forearms than the cryptic species echolocating at 98 kHz. However, no differences were detected in either wing morphology or diet, suggesting that there is limited resource partitioning between the two species. Thabah et al. (2006) suggested that the differences in echolocation calls are possibly due to character displacement on secondary contact to facilitate intra-specific communication. Studies on cryptic *Pipistrellus* spp. have also concluded that differences in echolocation calls may function for communication, not detection of different sizes of prey (Jones & Barlow 2004). Ecological studies of the mechanisms involved in resource exploitation, especially among morphologically similar species, are therefore needed so we can start unravelling the processes involved in the niche evolution of bats (Aldridge 1986, Saunders and Barclay 1992).

1.3.2. Cryptic species as an ecological tool

Cryptic species are defined as species which are identical in outward appearance or very nearly so, but are reproductively isolated (Allaby 1996). There has been some confusion in the literature however, of what defines a cryptic species, this is discussed by Bickford et al. (2006). Due to the recent developments research on cryptic animal species has increased exponentially over the last 20 years (Bickford et al. 2006). Jones and Barlow (2004) described 13 pairs of cryptic bat species, but with the aid of further molecular analysis, several other pairs have been discovered since then (e.g. Ibáňez et al. 2006, Mayer et al. 2007). Refer to Mayr (1970) for further information on cryptic species.

Sympatric cryptic species are simplified subsets of bat communities (Mayr 1977) and recently a number of studies have focused on their ecology (e.g. Arlettaz et al. 1997, Barlow 1997, Arlettaz 1999, Zhang et al. 2006). In fact, studies have shown that cryptic bat species occurring in sympatry forage in different habitats and have different diets. Barlow (1997)

showed that the two recently discovered cryptic Pipistrelle species, *Pipistrellus pipistrellus* and *P. pygmaeus* have different diets. Both species eat mainly small dipteran flies, but *P. pipistrellus* was found to eat substantially more insects from families of larger Diptera. A later study by Barlow et al. (1997) suggested that *P. pipistrellus* having a diet of larger prey may be due to its larger overall skull size, longer jaw, larger gape and longer upper canines. Vaughan et al. (1997), Russ and Montgomery (2002), Davidson-Watts et al. (2006) and Nicholls and Racey (2006b) found that the two species also had different habitat use. *P. pipistrellus* foraged in a range of habitats while *P. pygmaeus* fed mainly in riparian habitats. Arlettaz et al. (1997) and Arlettaz (1999) found in a Swiss study of the cryptic species *Myotis myotis* and *M. blythii* that these species also had very different habitat use and diets. *M. myotis* forage in freshly cut meadows and forests with no undergrowth, habitats with high accessibility to ground dwelling prey (e.g. carabid beetles). *M. blythii* on the other hand, forage in grassland (e.g. unmown meadows and pastures) with bush crickets being their most important prey. In North America, studies by Herd and Fenton (1983) on *Myotis lucifugus* and *M. yumanensis* and studies by Saunders and Barclay (1992) on *M. lucifugus* and *M. volans*, showed that resource partitioning occurs between both pairs of cryptic species through differences in habitat use.

Previous ecological studies of cryptic bat species have only looked at bats with similar evolutionary histories. Interestingly, a study carried out in 2001 on a great number of bat species, discovered that unlike the other study animals, the cryptic species Brandt's bat (*Myotis brandtii*) and the whiskered bat *(M. mystacinus*) have different evolutionary histories and are more closely related to other *Myotis* species than each other (Ruedi and Mayer 2001). While *M. brandtii* belongs to a clade together with a number of American bat species, *M. mystacinus* belongs to a clade with uncertain origins (Figure 1.1). The study also concluded that *M. mystacinus* and *M. brandtii* had a genetic sequence divergence of 16%, compared to e.g. under 2% for *Eptesicus serotinus* and *E. nilsonii*. *M. mystacinus* and *M. brandtii* may therefore show greater ecological differences than other cryptic bat species previously studied and therefore give us the perfect opportunity to study the outcomes of convergent evolution. These species make us able to challenge the ecomorphological paradigm, which states that bats with similar morphologies also have similar ecologies (see section 1.3.1). Additionally cryptic species give us an opportunity to reveal some of the processes involved in the niche evolution for bats, which may then be adapted further to whole guilds and communities of bats getting us one step closer to revealing the evolution of niches in bat communities.

Bickford et al. (2006) in a review paper of cryptic animal species conclude that further research should be carried out on cryptic species in order to resolve a range of ecological and evolutionary processes.

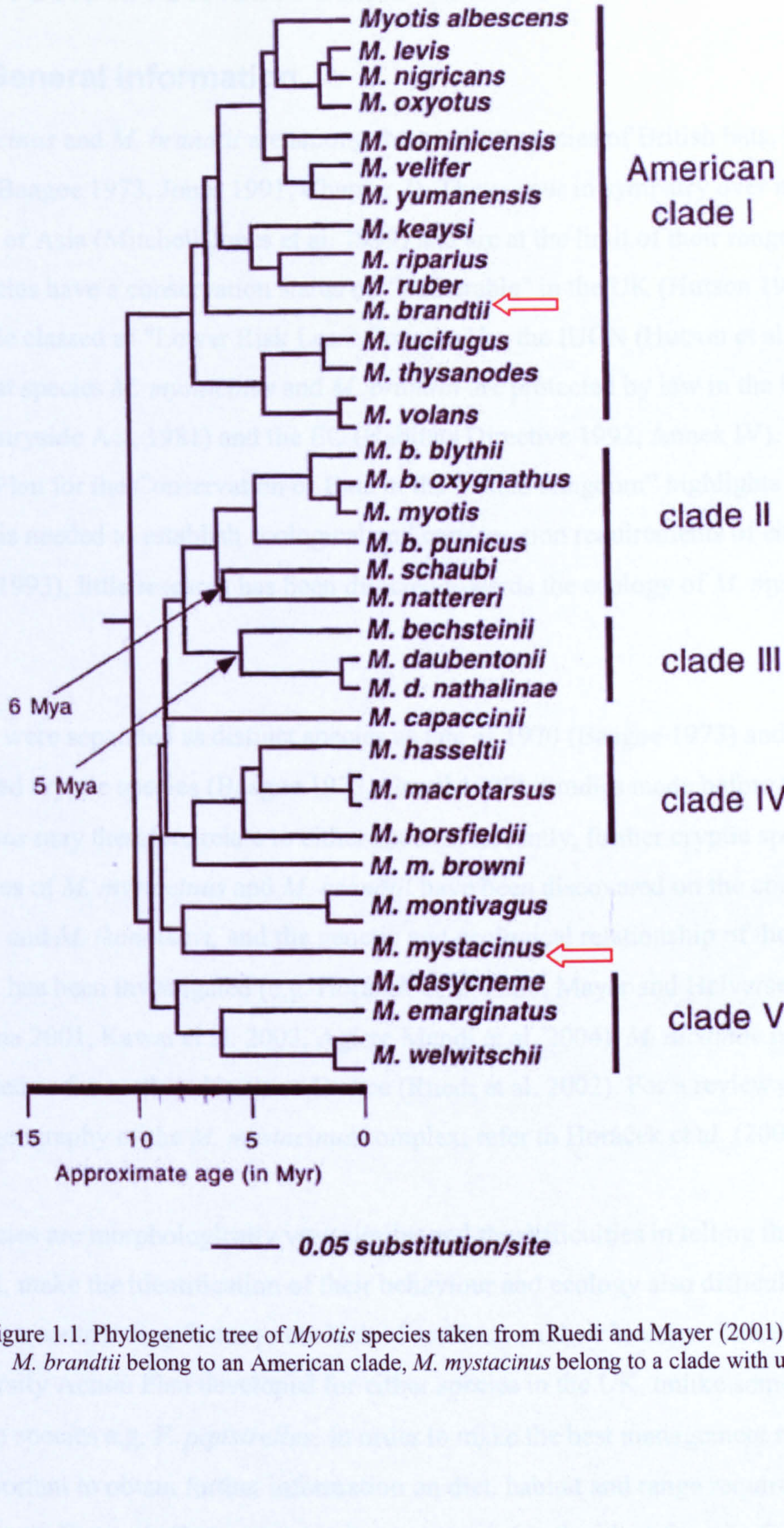

Figure 1.1. Phylogenetic tree of *Myotis* species taken from Ruedi and Mayer (2001) showing that while *M. brandtii* belong to an American clade, *M. mystacinus* belong to a clade with uncertain origins.

1.4. *MYOTIS MYSTACINUS* AND *M. BRANDTII*

1.4.1. General information

M. mystacinus and *M. brandtii* are among the smallest species of British bats, weighing only 3.5- 9 g (Baagøe 1973, Jones 1991, Chapter 2). They occur in sympatry over much of Europe and parts of Asia (Mitchell-Jones et al. 1999) and are at the limit of their range in the UK. Both species have a conservation status of "Vulnerable" in the UK (Hutson 1993), but are worldwide classed as "Lower Risk Least Concern" by the IUCN (Hutson et al. 2001). Like all British bat species *M. mystacinus* and *M. brandtii* are protected by law in the UK (Wildlife and Countryside Act, 1981) and the EC (Habitats Directive 1992, Annex IV). Although the "Action Plan for the Conservation of Bats in the United Kingdom" highlights that "further research is needed to establish ecological and conservation requirements of either species" (Hutson 1993), little research has been directed towards the ecology of *M. mystacinus* and *M. brandtii*.

The bats were separated as distinct species as late as 1970 (Baagøe 1973) and are now considered cryptic species (Baagøe 1973, Gerell 1987). Studies made before the 1970s on *M. mystacinus* may therefore relate to either species. Recently, further cryptic species and subspecies of *M. mystacinus* and *M. brandtii* have been discovered on the continent, e.g. *M. alcathoe* and *M. ikonnikovi,* and the genetic and ecological relationship of the *M. mystacinus* complex has been investigated (e.g. Horáĉek et al. 2000, Mayer and Helversen 2001, Tsytsulina 2001, Kawai et al. 2003, Agirre-Mendi et al. 2004). *M. alcathoe* has recently been discovered as far north as Northern France (Ruedi et al. 2002). For a review of the taxonomy and biogeography of the *M. mystacinus* complex, refer to Horáĉek et al. (2000).

The species are morphologically very similar and the difficulties in telling them apart, even in the hand, make the identification of their behaviour and ecology also difficult. This is probably a contributing factor to the lack of research on their biology. There is at present no Biodiversity Action Plan developed for either species in the UK, unlike some of the more common species e.g. *P. pipistrellus*. In order to make the best management recommendations it is important to obtain further information on diet, habitat and range requirements of the bat populations. Currently there are great inconsistencies in the identification features recommended for separating *M. mystacinus* and *M. brandtii* and frequently identification is not taken further than lumping the species as *M. mystacinus/brandtii*. Valuable information on

their ecology is consequently often lost or may even be inaccurate due to incorrect identification.

1.4.2. Roosting

1.4.2.1. Summer roosts

Both species roost mostly in buildings and more rarely in trees in Scandinavia, continental Europe and the UK (Schober and Grimmberger 1989, Zahn and Rupp 2004). A Finnish study found *M. mystacinus* using 75% manmade structures (Nyholm 1965). The bats have also been reported to roost occasionally in bat boxes and bridges (Schober and Grimmberger 1989).

Colonies of both species may use separate parts of the same roof (Gauckler and Kraus 1970) and may also roost together with *Pipistrellus* spp. or *Plecotus auritus* (Nyholm 1965, Gauckler and Kraus 1970). Usually there are less than 100 individuals roosting together. A study in northern England found a mean of 23.3 individuals in maternity roosts of *M. mystacinus* (n= 15) and 28.3 individuals in maternity roosts of *M. brandtii* (n= 5) (Jones and Altringham 1996).

1.4.2.2. Winter roosts

Both species hibernate in disused mines and caves and start their hibernation period in December. *M. brandtii* are usually found in warmer areas further away from the cave entrance than *M. mystacinus*, they also choose less humid situations. Note that *M. brandtii* males have been found to hibernate for longer then *M. mystacinus* males, with the end of hibernation typically occurring in May and March respectively (Jones 1991). Both species are occasionally found hibernating in cellars (Schober and Grimmberger 1989).

1.4.3. Social behaviour and organisation

1.4.3.1. Behaviour and social organisation during the summer

In both species adult males seem to be solitary (or occur in small groups) while adult females form nursery colonies to give birth and raise young.

In Finland, there was found to be two distinct periods of activity of *M. mystacinus* in the summer- after sunset and before sunrise. There were also intervals during the night when the bats would hang off a tree trunk, dry branch stump, vertical rock wall or live branch. The

lengths of these breaks were irregular (Nyholm 1965). A German radiotracking study of 9 *M. brandtii* females showed that the bats had one single period of activity from dusk until dawn. Weather conditions had almost no influence on the bats. The main hunting grounds were within 1.5 km to over 10 km away from the maternity colony (Dense and Rahmel 2002). Both species frequently fly along a regular beat or flight path at their foraging grounds (Schober and Grimmberger 1989).

The bats are usually sedentary, but the longest movement recorded is 1936 km, south west from Russia to Bulgaria (Krzanowski 1961) for *M. mystacinus* and 230 km for *M. brandtii* (Schober and Grimmberger 1989).

Both species can be found swarming at underground sites from August until October, with *M. brandtii* having a peak in early August and *M. mystacinus* swarming throughout most of the swarming season, from early August until middle of October (Parsons et al. 2003). The purpose of swarming is not fully understood, but mating or information transfer have been put forward as possible explanations (Parsons et al. 2003). In a disused limestone mine in south west England 80% of the captured *M. mystacinus* and 60% of the captured *M. brandtii* were males (Parsons et al. 2003).

1.4.3.2. Behaviour and social organisation during the winter

M. mystacinus and *M. brandtii* have been observed flying in daylight in the winter and spring (Schober and Grimmberger 1989). Winter movements of 2.5 km have been recorded for *M. brandtii* in Suffolk (R.E. Stebbings unpublished).

1.4.4. Habitat use

M. mystacinus and *M. brandtii* have wing morphologies and echolocation calls which indicate that they forage along woodland edge or in moderately cluttered habitats (Norberg and Rayner 1987). The only study on habitat use that focuses on both bat species was carried out in Germany by conducting habitat surveys around known maternity colonies. The study concluded that the principal foraging habitat of *M. brandtii* was deciduous woodland with particularly damp areas, close to water (Taake 1984). Coniferous woodland, woodland edge and clearings were also selected by *M. brandtii*, but there is some disagreement in the literature on the importance of coniferous woodland to the bats (e.g. Taake 1984, Ekman and DeJong 1996, Johansson and DeJong 1996). The same German study reports that *M.*

mystacinus had a weaker connection to wooded areas and most frequently selected parks, gardens and villages (Taake 1984). *M. brandtii* is therefore generally believed to be a forest bat, while *M. mystacinus* is more a bat of the open country. However, when considering these results, we have to keep in mind that this study was not carried out using radiotracking or acoustic surveys, but rather a survey of habitats surrounding maternity colonies.

1.4.5. Diet

The diet of *M. mystacinus* and *M. brandtii* is also poorly documented with only one published study on the diet of *M. brandtii*. This German study concluded that the bats have similar diets, mostly comprised of Lepidoptera and Diptera (Taake 1992, 1993). However, Lepidoptera are generally thought to be over represented in dietary studies of bats (Robinson and Stebbings 1993). Araneida were also found frequently, suggesting a gleaning habit (Taake 1992, 1993). The diets of the two species were found to be similar also down to family level (Taake 1992, 1993).

1.5. OBJECTIVES AND THESIS OUTLINE

1.5.1. Question

What are the mechanisms for resource partitioning between two species that are morphologically almost indistinguishable, but have different evolutionary histories?

1.5.2. Thesis aims and objectives

1) To determine whether *M. mystacinus* and *M. brandtii* have similar foraging ecologies and behave as predicted by their ecomorphology. This will be achieved by comparing the diet, habitat use, nocturnal activity and morphology of the two species.

2) Critically, to test whether identification according to morphological criteria used in the field (e.g. dentition, pelage colour and penis shape) corresponds with identification based on gene sequencing. This will be achieved by genetically verifying species identification and statistically testing which morphological features show the least degree of overlap.

1.5.3. Thesis organisation

In **Chapter Two,** I present the best morphological features for distinguishing between *M. mystacinus* and *M. brandtii* based on gene sequencing.

In **Chapter Three,** I present a habitat use study of *M. mystacinus* and *M. brandtii*.

In **Chapter Four,** I present data on the nocturnal activity of *M. mystacinus* and *M. brandtii*.

In **Chapter Five,** I present results on the dietary differences between *M. mystacinus* and *M. brandtii*.

Chapter 6 is a general discussion, ending with conclusions and future recommendations from the study.

Chapter 7 is the reference section.

2. MORPHOLOGICAL DIFFERENCES AND IDENTIFICATION FEATURES

ABSTRACT

There is currently great confusion and inconsistencies in how to distinguish between *M. mystacinus* and *M. brandtii*. This sometimes leads to identification not being taken further than combining the species as *M. mystacinus/brandtii*. Valuable information on the bats' ecology may therefore often be lost or inaccurate due to incorrect identification.

The most commonly used identification features are penis shape, tragus shape, colouration of pelage/ wing membrane and teeth shape/ length. However, the reliability of these features for identification has never been tested using specimens whose identity is unequivocal.

The aim of the project was to test whether commonly used identification features correspond with identification based on gene sequencing. Additionally, I tested a range of additional features looking for an easier and more reliable way of identifying *M. mystacinus* and *M. brandtii* in the field. Although morphologically very similar, these species are genetically very divergent allowing positive identification from sequences of the mitochondrial Cytochrome *b* gene.

All the morphological features tested showed more or less overlap between the two species. It is therefore recommended to use a combination of several features when distinguishing between *M. mystacinus* and *M. brandtii*. Penis shape, upper jaw dentition, lower jaw dentition, tragus shape and thumb claw length used in combination were statistically the best features for distinguishing between *M. mystacinus* and *M. brandtii,* classifying 100% of the bats to the correct species.

2.1. INTRODUCTION

2.1.2. Genetics as a taxonomical tool

DNA sequence analysis has proven to be a powerful tool in the detection of cryptic species (e.g. Barratt et al. 1997, de Vargas et al. 1999, Castella et al. 2000, Yoder et al. 2000, Mayer and Helversen 2001, Kiefer et al. 2002, Ibáñez et al. 2006, Mayer et al. 2007) because genetic differences between species accumulate with time while this is not necessarily true for morphological characters. For example a cryptic species within the *Myotis mystacinus* complex (*M. alcathoe*) was identified using a combination of morphological, echolocation and molecular data (sequences from the ND1 gene) (Helversen et al. 2001). Another cryptic species of the *M. mystacinus* complex (*M. aurascens*) was separated from *M. mystacinus* by a small number of morphological characters (Benda and Tsytsulina 2000), but analysis of mitochondrial DNA (the complete ND1 gene) was unable to support this (Mayer and Helversen 2001). The taxonomic status of *M. aurascens* is still uncertain (Dietz and von Helversen 2004). Mayer et al. (2007) have recently suggested that a total of 51 bat species can be distinguished according to DNA sequence analysis, rather than the 37 morphologically defined vespertilionid bat species previously acknowledged for the western Palaearctic.

Mitochondrial DNA (mtDNA) accumulates mutations rapidly, thus providing a number of genotypic characters when analyzed by PCR (polymerase chain reaction) and nucleotide sequencing (Kocher et al. 1989, Lanyon et al. 1992) and has consequently been widely used in phylogenetic studies. The Cytochrome *b* gene is one of the most commonly used regions of mtDNA because the mutation rate at silent positions is relatively fast, whilst the mutation rate in terms of non-synonymous substitutions is slow (Irwin 1991). A range of molecular studies have used the Cytochrome *b* gene to analyse both inter- and intra-specific relationships among bat species (e.g. Barratt et al. 1997, Kawai et al. 2003, Juste et al. 2004, Stadelmann et al. 2004, Harris 2006, Ibáñez et al. 2006, Mayer et al. 2007). Cytochrome *b* has been shown to be a good marker for identifying cryptic species within the genus *Myotis* (e.g. Piaggio et al. 2002, Bickham et al. 2004). Even though this technique is not yet useful for application in the field, genetic information can be used to evaluate groupings based on other characters, for example morphological features for the identification of cryptic species.

2.1.2. Distinguishing between *Myotis mystacinus* and *M. brandtii*

Ecological studies show that cryptic bat species often have different ecologies (e.g. Taake 1984, 1992, 1993, Arlettaz 1996, Arlettaz et al. 1997, , Barlow 1997, Arlettaz 1999, Davidson-Watts and Jones 2006, Davidson-Watts et al. 2006, Nicholls and Racey 2006a, 2006b, Chapter 3, 4, 5) and consequently need different management plans. However, in order to carry out any ecological study researchers need to be able to identify their study animals with confidence.

The cryptic species *M. mystacinus* and *M. brandtii* have very similar morphologies, making identification, even in the hand difficult. This, in addition to disagreements in the literature on appropriate identification features lead many bat workers into lumping the two species together as *M. mystacinus/brandtii*. There is currently no Species Action Plan for either species even though they are classified in the UK as "vulnerable to extinction" (Hutson 1993) and little research has been made on their ecologies. Further ecological studies to establish the correct management for conservation are therefore critical (Hutson 1993) in addition to morphological studies to identify reliable identification features.

The most commonly used identification features to distinguish between *M. mystacinus* and *M. brandtii* are shape of the penis and tragus, colouration of pelage, ear and wing membrane and dentition. Since the variations are generally small, and most identification takes place in the field at night, it is needless to say that these identification features are not easy to use. Additionally, the features' reliability for identification has never been tested.

My aim was therefore to critically, test whether identification according to morphological criteria (e.g. dentition, pelage colour and penis shape) determined under field conditions correspond with identification based on gene sequencing.

2.2. METHODS

2.2.1. Capture and processing of bats

77 bats provisionally identified as *M. mystacinus* and 65 bats provisionally identified as *M. brandtii* were captured using harp traps (2.4 x 1.85 m, Faunatech Austbat, Victoria, Australia)

at the entrance of two disused limestone mines (Box limestone mine and Byfield mine) near Bath in Wiltshire, South West England, from August until October during 2003 and 2004. The harp traps were checked every 30 minutes.

Captured bats had a range of standard biometric and morphological measurements taken. Continuous variables were recorded by using colour codes and categories predetermined from a pilot study (Table 2.1). All bats were weighed to the nearest 0.1 g, by using a 30 g Pesola spring balance and had measurements taken to the nearest 0.1 mm by using a set of dial callipers. Measurements were carried out according to Table 2.2. Animals were then sexed and reproductive condition of females was assessed according to Anthony (1988) and Hutson and Racey (2004). An outline of the bats' right wing was drawn and later digitised using the software Digitize (Rockware Inc., Colorado, USA) and a magnetic tablet (SummaSketch III; Summagraphics, Seymour, Connecticut, USA) so that the aspect ratio, wing loading and tip shape index could be calculated according to Norberg and Rayner (1987). A 3 mm biopsy punch (Stiefel Laboratories, distributed by Schuco International, London Ltd, UK) was taken from the plagiopatagium and stored in 100% ethanol (Worthington Wilmer and Barratt 1996). The bats were then released at the mine entrance as quickly as possible. All measurements and recordings were made by the same researcher.

2.2.2. Molecular analysis

Molecular analysis was carried out according to Harris (2006), as described below.

2.2.2.1. DNA extraction

To eliminate ethanol carry-over, the wing tissue was washed in sterilised, distilled water and air-dried. DNA was extracted using the DNeasy Tissue Kit (Qiagen, UK) according to the manufacturer's "Protocol for Animal Tissues". This included the samples being lysed overnight using proteinase K and then loaded onto DNeasy Mini spin columns. The DNA is selectively bound onto the column membrane by centrifugation after contaminants and enzyme inhibitors have been removed. The DNA was then eluted into 200 µl elution buffer. 10 µl DNA was finally run in 2% agarose gel in a gel electrophoresis apparatus (Mini horizontal gel electrophoresis tank, SciPlas HU6 with Consort E443 power supply) and visualised using a UV transilluminator (BioDoc-It gel imaging system) to ensure successful extraction.

2.2.2.2. Primers

Initially the primer pair BarbF1, forward primer (5'- CCT CAA ATA TTT CAT CAT GAT-3') and BarbR2, reverse primer (5'- GTC CTC CAA TTC ATG TTA GGG -3') were used for amplification. This primer pair is a set of universal primers designed to amplify DNA from most UK bat species (Harris 2006). For further information on primer design, refer to Harris (2006). However, these primers did not amplify DNA for *M. mystacinus* for unknown reasons. Consequently, S. Harris designed two primer pairs (forward and reverse), one primer pair exclusively to amplify DNA from *M. mystacinus:* WF, forward primer (5'- CCTGCCCCATCAAATATCTC -3') and WR, reverse primer (5'- GGAATTGATCGTAGGATTGCG -3') and one primer pair to amplify DNA from both *M. mystacinus* and *M. brandtii:* BWF, forward primer (5'- GACCAACATTCGAAAATC -3') and BWR, reverse primer (5'- GTGATGCTGCGTTGTTTG -3'). All three primer pairs were used to amplify DNA from the same template DNA separately in order to maximize the number of successful amplications. All three primer pairs amplify a 900 bp length of the Cytochrome *b* gene between 71- 1021 bp.

2.2.2.3. Polymerase chain reaction (PCR) and PCR product detection

Optimisation for all three primer pairs included varying concentrations of template DNA and testing different amounts of Magnesium Chloride ($MgCl_2$) in the master mix. The annealing temperature for the first primer pair (BarbF1 and BarbR2) had been optimised by Harris (2006). The remaining two primer pairs were tested on gradient PCRs on a gradient thermal cycler (Peltier Thermal Cycler-Gradient Cycler DNA Engine PTC-200) to find the optimal annealing temperature (BWF and BWR tested between 52- 58 °C, WF and WR tested between 42- 48 °C).

10 µl of template DNA was added to aliquots of 45 µl master mix (Box 2.1). Thermocycling conditions for BarbF1 and BarbR2 are described in Box 2.2. Similar thermocycling conditions were used for BWF, BWR, WF and WR, except from an annealing temperature of 56.5 °C for BWF and BWR and 45.6 °C for WF and WR. A gradient thermal cycler (Peltier Thermal Cycler-Gradient Cycler DNA Engine PTC-200) was used for all amplifications. Agarose gel electrophoresis of PCR products was as described in section 2.2.2.2. For PCR product size comparisons a 2- Log DNA ladder (New England BioLabs Inc.) was loaded on the agarose gel alongside the DNA samples.

2.2.2.4. Sequencing of PCR products

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, UK) following the manufacturer's guidelines where 20 µl PCR product was eluted in 30 µl Buffer EB. The sequencing reaction master mix consisted of 11 µl of purified PCR product, 1 µl forward or reverse primer (0.1 µM) and 8 µl of ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (ABI, UK). For thermocycling conditions, which were similar for all 3 primers pairs, refer to Box 2.3.

2.2.2.5. Precipitation of sequence extension products

An isopropanol method was used for precipitation of extension products. 20 µl sequencing product was added to isopropanol and spun repeatedly, with the supernatant being removed and more isopropanol added between each spin. After the final spin, the supernatant was removed, taking care not to remove the pellet that had adhered to the side of the tube. The pellet was then air dried and samples were stored at -20°C.

2.2.2.6. Automated DNA sequencing

Samples were sent to Lark Technologies (Essex, UK) for automated DNA sequencing using an ABI PRISM 3100 Genetic Analyser.

2.2.2.7. Sequence analysis

Editing and trimming of sequences were carried out using BioEdit 7.0.5.3 and ChromasLite 2.01. Sequences were then compared with *M. mystacinus* (GenBank accession number AF376861) and *M. brandtii* (GenBank accession number AF376844) sequences available from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) in a multiple sequence alignment. A homology of >98% with the Genbank sequences, including any Ns, in a 150 bp sequence was determined as acceptable for species identification because the sequence divergence between *M. mystacinus* and *M. brandtii* is large, 16% and 19.6% according to Ruedi and Mayer (2001) and Harris (2001) respectively. Additionally a pairwise sequence alignment showed only 6 conserved regions between the reference sequences, the longest being 22 bp (settings: minimum 15 bp, 0.2 max entropy, gaps limited to two per segment). All bats had previously been identified in the hand by the researcher as *M. mystacinus* or *M. brandtii* consequently there was no risk of species other than *M. mystacinus* complex species being present in the genetic samples.

2.2.3. Statistical analysis of identification features

Only the males were included in the statistical analysis due to differences in the numbers of sequenced females for each species. Female *Myotis* bats are often slightly larger than males (e.g. Benda 1994, Jones and Kokurewicz 1994, Gaisler and Zukal 2004) so including them in the statistical analysis could have influenced the results. Ear length was not included in the statistical analysis because some of the measurements were inaccurate due to the bats folding their ears.

When each bat was identified correctly to species, a Mann- Whitney U test was carried out on the continuous variables to test for significant differences between the species. This test was chosen over ANOVA after an Anderson- Darling test showed that some of the data were not normally distributed. Similarly, a Chi- square test was carried out on the categorical variables to test for differences between the species. Multiple comparisons adjustment was carried out as suggested by Benjamini and Hocberg (1995). Variables that were not statistically significant i.e. no difference between the two species, were not included in further analysis. The remaining variables were put into a logistic regression model. The most significant variables from the logistic regression model were then included in a backward stepwise logistic regression model. Some significant variables were not included in the model because the sample size was too small to include a large number of variables. Ideally one should aim for a sample size of 10 for each variable tested. If the sample size is small and the number of variables large, there is a greater chance of bias, however Choi and Howe (1984) concluded from a computer simulation used to evaluate the performance of logistic regression models that the power of the test is altered little with sample sizes and numbers of variables differing from the recommended numbers. The variables that remained in the model were then put separately into logistic regression models. Only characters found in both sexes were analysed in the same model. Minitab 14 and SPSS 12.0 were used for statistical analysis with a significance level of 5%.

2.2.4. Statistical analysis of wing morphology

Due to differences in numbers of sequenced females between the two species, again only males were included in the statistical analysis (refer to section 2.2.3). A Mann- Whitney U test was chosen over ANOVA to test for significant differences between the wing morphological variables of the two species after an Anderson- Darling test showed that some

of the data were not normally distributed. Multiple comparisons adjustment was carried out as suggested by Benjamini and Hocberg (1995). The most significant variables were then put into a logistic regression model. Minitab 14 and SPSS 12.0 were used for statistical analysis with a significance level of 5%.

2.3. RESULTS

2.3.1. DNA extraction

From the original number of DNA extractions that were attempted (n= 124), 88 samples were successfully extracted. Therefore, not all samples were taken to the PCR stage.

2.3.2. PCR

After optimising the master mix and the programme, 80 samples (out of 88 samples with successful DNA extraction) were successfully amplified by PCR and sent off for automated DNA sequencing.

2.3.3. Sequence analysis

80 sequences were edited and trimmed. 33 of the sequences were of sufficient quality for further analysis, 16 of the sequences were identified as *M. mystacinus* and 17 as *M. brandtii.* The remaining 47 sequences were either of poor quality or had been contaminated. Aligned sequences of reference sequences, *M. mystacinus* and *M. brandtii* can be seen on Plates 2.1-2.3.

2.3.4. Univariate statistical analysis of identification features

There is overlap for all continuous variables, but *M. mystacinus* is generally smaller than *M. brandtii* (Table 2.3 and 2.4). The continuous variables were analysed using the Mann-Whitney U test on males only (Table 2.5) and tragus length, calcar length, foot length and tragus width did not differ significantly between the two species. Body mass however and 5th digit length were statistically significant ($U=78.0$, $N_1= 16$, $N_2= 17$, $P= 0.0426$), but the multiple comparison adjustment showed insignificant results, they were therefore not considered for further analysis. The multiple comparison adjustment made no difference to the significance of the other variables.

The categorical variables were analysed for males only using the Chi Square test (Table 2.6). Ventral pelage colour, dorsal pelage colour, penis colour and ear colour did not differ significantly between the species. A multiple comparison adjustment did not change the statistical results. A breakdown of the categorical variables for each species can be seen on Table 2.7 and 2.8.

2.3.5. Multivariate statistical analysis of identification features

The variables that were found to be suitable for further analysis were put into a logistic regression model (Table 2.9). Results for the logistic regression model were X^2= 20.333, d.f. 12, P= 0.061. The most significant variables were then put into a backward stepwise regression model (refer to section 2.2.3. for further explanation) where foot claw length and thumb length were automatically removed by the statistics software. The remaining variables: lower jaw dentition, upper jaw dentition, tragus shape and thumb claw length could separate 100% of the bats in the model (X^2= 31.492, d.f. 7 P= 0.000). The remaining variables in the model were then put into logistic regression models separately (Table 2.10). No single feature or a combination of two or three features could separate the two species with 100% certainty, which indicates some degree of overlap in all features (Tables 2.7, 2.8, 2.9). The degree of overlap for upper jaw dentition, lower jaw dentition, tragus shape, penis shape and thumb claw length can be seen in Figures 2.1a-e respectively showing that there is some overlap on all features.

2.3.6. Univariate statistical analysis of wing morphology

There is little difference in all wing morphology variables between the two species, but *M. brandtii* is generally significantly larger than *M. mystacinus* (Table 2.11 and 2.12). Particularly, *M. brandtii* have a slightly higher aspect ratio. The variables were analysed using the Mann-Whitney U test on males only, but only forearm length (U=78.0, N_1= 16, N_2= 17, P= 0.010) and body mass (U=88.0, N_1= 16, N_2= 17, P= 0.0426) differed significantly between the two species. However, a multiple comparison adjustment showed that these results were insignificant.

2.3.7. Multivariate statistical analysis of wing morphology

The most significant variables (body mass, wing area, forearm length) were then put into a logistic regression model. The results for the logistic regression model were X^2= 3.926, d.f. 13, P= 0.27. The model could only separate 65.2% of the bats to the correct species.

2.4. DISCUSSION

2.4.1. Identification

2.4.1.1. Dentition

Dentition can be a difficult feature to use for identification because the bats are small and researchers normally work under bad light. Baagøe (1973) however, concluded that dentition was the only reliable way of distinguishing between female *M. mystacinus* and *M. brandtii*. Statistically the best identification feature in my study was upper jaw dentition with an overall classification rate of about 91%. The difference lies in the presence or absence of a cusp or protocone on the 4th upper premolar (the cusp is absent or smaller than the 3rd upper premolar in *M. mystacinus* and the same height or larger than the 3rd upper premolar in *M. brandtii*, see Plate 2.4). Lower jaw dentition was also found to be a good identification feature with an overall classification rate of 81%. In the lower jaw the difference lies in the ratio of lower premolar 2 to lower premolar 3. *M. mystacinus* have lower premolar 3 less than half the height of premolar 2, while in *M. brandtii* premolar 3 is more than half the height of premolar 2 (Plate 2.5). However, both species can have premolar 3 very close to half the height of premolar 2, making identification problematic. Note that Baagøe (1973) also states that dentition is only reliable as a distinguishing feature when looking at the dentition in the lower jaw, not the upper jaw. However, the bats used by Baagøe were not identified by molecular methods and may consequently have been misidentified. It is also important to look at the lower and upper jaw dentition in both sides of the jaw in individual bats as they may occasionally differ.

2.4.1.2. Penis shape

It has generally been believed that penis shape is a very good feature to distinguish between *M. brandtii* and *M. mystacinus*. There is some verbal disagreement whether this is also true for sub-adults. *M. mystacinus* have been believed to have a thin and straight penis, while *M. brandtii* have been thought to have a bell or club shaped penis (Plate 2.6). However, my results show that penis shape is not always a reliable identification feature. While all the *M. mystacinus* males had a thin penis, just over 30% of the *M. brandtii* males had a thin penis and just under 70% had a club shaped penis. It is therefore not completely safe to assume that

a bat with a thin penis is M. mystacinus. However, if the bat has a club shaped penis, it is reasonable to assume it is M. brandtii. Note that the M. brandtii with a thin penis were adults.

2.4.1.3. Tragus shape

Tragus shape could be used to distinguish between the two species with 82% certainty and is therefore a good identification feature. While *M. brandtii* has a tragus with a convex posterior edge, *M. mystacinus* has a tragus with a concave or straight posterior edge (Plate 2.7).

2.4.1.4. Length of thumb claw

Length of the claw on the thumb could distinguish between the two species with 91% overall certainty. However, there is some overlap. *M. mystacinus* had thumb claw lengths ranging from 1.2- 2.1 mm, while *M. brandtii* had thumb claw lengths between 1.5- 2.3 mm. This feature can therefore only be used for the regions with no overlap i.e. for bats with very short or very long thumb claws. Additionally, making accurate measurements of claws can be difficult under field conditions.

2.4.1.5. Using the identification features

There are no published studies on the reliability of the identification features used to distinguish between *M. mystacinus* and *M. brandtii* based on gene sequencing. In my study, no single feature was found that could discriminate between the two species with 100% certainty. It is important to keep in mind that this is a fairly small sample size so we have to be careful when drawing conclusions as to which features are better for identification. On the other hand, even with such a small sample size we can still see a trend in that there is more or less overlap for all identification features. However, *M. mystacinus* is generally smaller then *M. brandtii* for all continuous variables.

I suggest that the best way of distinguishing between the two species is by using a combination of upper jaw dentition, penis shape, tragus shape, thumb claw length and lower jaw dentition. Identification should then be based upon how many features correspond with each species. However, until a feature with no overlap between species has been detected or identification can be verified using molecular methods, all field identifications of *M. mystacinus* and *M. brandtii* should be regarded with some caution. On the other hand, it is still important to keep in mind that the five features mentioned above, used in combination, did

classify 100% of the bats correctly and that each of these features when used separately could classify over 80% of the bats to the correct species.

2.4.1.6. Future work

Some additional morphological features may be useful for identification. These features were not tested either due to a lack of suitable equipment or because their potential only became apparent too late into the project.

Kawai et al. (2006) found that a difference in a major blood vessel in the uropatagium is a reliable feature for distinguishing between *M. brandtii gracilis* and *M. ikonnikovi*. Ruprecht and Yablokov (1977) found significant differences in patagium venations between *Plecotus auritus* and *P. austriacus*. Haussler et al. (1999) found differences in the position of the auricles (eye sockets) and shape of the nostril pad of *Pipistrellus* spp., while Dietz and von Helversen (2004) recommend looking at the notch of the posterior edge of the ear compared to tragus length to distinguish between the *M. mystacinus* complex species. Slope of the forehead is said to be used by Hungarian bat workers to distinguish between *M. mystacinus* and *M. brandtii* (Phil Richardson pers. comm.). Tibia length, digit length, metacarpal length and length of phalanges may also be useful for identification. Also, additional dental characteristics and characteristics of individual hairs in their pelage may be useful for identification of bats. Hair characteristics are particularly interesting since it could be used for distinguishing between the species in roosts without the need of handling the bats. All the features mentioned above should therefore be tested. Additionally, the five features best suited for identification, as described in section 2.4.1.5 (upper and lower jaw dentition, tragus shape, penis shape and thumb claw length), should be tested on a larger sample of both sexes and of bats from different geographic areas, both in the UK and throughout their distribution range.

2.4.1.7. Additional taxa in the *M. mystacinus* species complex

Due to great intra-specific variation in morphology there has been some discussion whether there are other *M. mystacinus* complex species present in the UK; either a new *Myotis* species or perhaps the sibling species *M. alcathoe*, which looks very similar to *M. mystacinus* and *M. brandtii* and has recently been found as close to the UK as northern France (Ruedi et al. 2002). There was no evidence of a third species among the 33 genetic sequences of good enough quality for analysis. However, note this is a very small sample size for such a study.

2.4.2. Wing morphology and its relation to ecological differences

Bats have been shown to be very flexible in their use of echolocation (Simmons et al. 1978, Fieldler 1979, Habersetzer 1981, Bell 1982, Vogler and Neuweiler 1983, 1984). It is therefore believed that the primary mechanism leading to resource partitioning in bat communities is wing morphology and therefore manoeuvrability, with echolocation being varied to suit a particular foraging situation (Fenton and Rautenbach 1986).

Aldridge (1986) demonstrated how manoeuvrability and foraging behaviour in insectivorous bats is influenced by wing morphology and O'Shea and Vaughan (1981) emphasised correlations between aspect ratio and foraging behaviour. However, for most insectivorous bats, flight ability, as constrained by wing morphology may be more important for prey capture than for habitat use. For example Saunders and Barclay (1992) did not successfully predict differences in ecology by using the wing morphology of *M. lucifugus* and *M. volans*. Additionally, because behaviour is so flexible, individual animals may behave in a way that a study of their morphology would not predict.

Wing morphology and echolocation calls indicate that *M. mystacinus* and *M. brandtii* are edge or cluttered habitat foragers (Norberg and Rayner 1987). However, my study indicates that there is a small difference in aspect ratio between the two species, although this difference is not statistically significant. There is no clear link between the morphology of the two species and the differences in their habitat use and foraging behaviour (Chapter 3, 4). However, the results may have been influenced to a degree by the small sample size of the study and the possibility of juveniles being identified as adults.

2.5. CONCLUSION

There are currently no published studies on the morphological differences between *M. mystacinus* and *M. brandtii* based on gene sequencing. The results in this chapter show that their morphologies are remarkably similar, so similar that in fact, all of the features tested showed more or less overlap. However, by using several morphological features, it is still possible to identify these two species with reasonable confidence.

Even with such high morphological similarity, the foraging ecologies of *M. brandtii* and *M. mystacinus* are different. Morphological similarity among recently diverged cryptic species can be explained by a lack of natural selection on morphological characters or a lack of time for morphological divergence. The latter is unlikely since *M. mystacinus* and *M. brandtii* are believed to have diverged around 10 mya (Ruedi and Mayer 2001).

Variable	Categories
Penis shape	Clubshaped, straight
Penis colour	Pink, light pink, pink with beige tip
Ear colour	Dark brown, medium brown
Wing membrane colour	Dark brown, medium brown
Face colour	Dark brown, medium brown, golden
Dorsal colour	Dark brown, medium brown, golden
Ventral colour	Golden brown, light golden brown, light brown, light grey brown, medium grey
Upper jaw dentition (P4 cusp size compared to P3)	Small, medium, large
Lower jaw dentition (Height of P3 compared to P2)	Small, medium, large
Tragus shape	Whiskered (concave or straight), Brandt's (convex)

Table 2.1. Categorical variables recorded for *M. mystacinus* and *M. brandtii*

Variable	Measurement
Body mass	Standard measurement
Forearm length	Standard measurement
Thumb length	Base of thumb to tip of claw
Thumb claw length	Base to tip of claw
Tragus length	Base to tip of tragus
Tragus width	Widest point of tragus
Penis length	Base to tip of penis
Ear length	Base to tip of ear
Foot length	Base of heel to tip of claw on middle toe
Foot claw length	Base to tip of claw on middle toe
5th digit length	Base to tip of 5^{th} digit
Calcar length	Base to tip of calcar with tail extended

Table 2.2. Continuous variables recorded for *M. mystacinus* and *M. brandtii*

Reagent	Quantity
HPLC H_2O	34.75µl
PCR buffer (10x)	5.00 µl
dNTPs (10 mM)	1.00 µl
Forward primer	1.00 µl
Reverse primer	1.00 µl
TAQ	0.25 µl
$MgCl_2$ (mM)	2.00 µl
Template DNA	5.00 µl
Total	50.00 µl

Box 2.1. Mastermix for PCR

Cycle 1	Initial denature	15 minutes at 95 °C	1 temperature x 1 cycle
Cycle 2	Denature	50 seconds at 95 °C	3 temperatures x 37 cycles
Cycle 2ii	Anneal	50 seconds at 50 °C	
Cycle 2iii	Extension	60 seconds at 72 °C	
Cycle 3	Final extension	10 minutes at 72 °C	1 temperature x 1 cycle

Box 2.2. Thermocycling conditions for primers BarbF1 and BarbF2

Denature	10 seconds at 96 °C	3 temperatures x 25 cycles
Anneal	5 seconds at 50 °C	
Extension	4 minutes at 40 °C	

Box 2.3. Thermocycling conditions for amplification of extension products

GACCAACATTCGAAAATCTCACCCCTTAATAAAAATTATTAACAGCTCATTTA
GACCAACATTCGAAAATCCCACCCCTTAGTAAAAATTATTAATAGCTCATTTA

TTGACCTCCCTGCCCCATCAAACATTTCATCTTGATGAAACTTTGGATCTCTCC
TCGACCTTCCTGCCCCATCAAATATCTCATCTTGATGAAATTTCGGATCTCTTT

TAGGAATTTGCTTAGCACTACAAATTTTAACAGGACTATTTCTAGCTATACAC
TAGGAATCTGCCTAATACTACAAATTCTGACAGGACTATTCCTAGCTATACAC

TACACATCAGACACCACAACAGCTTTTAACTCTGTCACCCATATTTGCCGAGA
TATACGTCAGA TACTGCAACAGCCTTCAACTCTGTTACCCATATCTGCCGAGA

TGTAAACTATGGTTGAGT TCTACGCTACTTGCATGCAAATGGAGCCTCCATAT
CGTAAATTACGGCTGAGTCCTACGCTACTTACATGCAAACGGAGCTTCTATAT

TTTTTATCTGCCTATATCTCCATGTAGGACGGGGCCTTTACTATGGGTCCTA T
TTTTCATTTGCCTATATCTTCATGTAGGACGAGGACTTTACTACGGATCCTAC

ATACAGAAACCTGAAATATCGGAGTTATTCTATTATTTGCTGTAATAGCATAATA
ATACAGAAACTTGAAATATTGGAGTAATCTTACTATTTGCTGTAATAGCAGCATA

ACAGCCTTTATAGGATATGTACTTCCATGAGGACAAATGTCTTTCTGAGGAGC
ACAGCTTTCATAGGATACGTACTCCCATGGGGCCAAATGTCCTTTTGAGGTGC

AACAGTAATTACCAACCTGCTCTCTGCAATTCCGTACATTGGAACAGACCTTG
AACCGTGATCACTAACCTACTCTCTGCCATTCC ATATATTGGTACAGACCTGG

TAGAATGAATCTGAGGCGGCTTCTCTGTTGACAAAGC TACTTTGACCCGATTC
TAGAATGGATCTGAGGAGGCTTCTCCGTTGACAAGGCCACCTTAACTCGATTC

TTTGCCTTTCACTTTTTACTCCCATTTATTATTGCAGCCATAGTCATAGTCCAC
TTTGCTTTCCACTTTCTACTCCCATTTATCATCTCCGCCATGGTCATAGTCCAT

CTCCTATTTCTTCACGAAACCGGATCCAATAACCCAACAGGAATCCCCTCCAA
CTTCTATTCCTTCACGAAACAGGATCTAATAACCCAACAGGAATTCCTT CTAA

CGCTGATATAATCCCCTTCCACCCCTACTATACAATTAAAGACATTCTTGGCC
TATAGACATAATTCCCTTTCACCCGTATTATACAATTAAAGA TATTTTGGGCC

TGCTATTAATAATTACAGTCCTACTCATACTAGTACTATTCTCCCCCGACCTG
TACTACTAATAATCACGGCCCTTCTAGTACTAGTACTATTTTCCCCCTGACATA

CTAGGAGACCCTGACAACTACACACCAGCGAACCCACTAAACACCCCCTCCCC
CTAGGAGACCCCGATAACTATATACCAGC AAATCCTCTAAACACCCCTCCTC

ATATCAAACCGGAATGGTACTTTTTATTCGCATATGCAATTCTACGATCAATT
ACATTAAACCAGAGTGATATTTTCTATTTGCATACGCAATCCTACGATCAATT

CCAAACAAACTAGGAGGAGTGTTAGCCCTAGTACTATCAATCCTTATTCTAAT
CCAAATAAATTAGGAGGAGTACTAGC TCTAGTCCTCTCTATCCTTATTCTAAT

TATCATTCCCCTACTCCACACCTCCAAACAACGCAGCATAACTTTTCGTCCCT
TATTATCCCCCTACTTCACACATCCAAACAACGCAGCATGACTTTCCGTCCCC

TAAGCCAGTGCCTATTCTGACTATTAACAGCAGATCTATTCACTCTAACATGA
TAAGTCAATG TTTATTTTGACTATTAGTAGCAGATCTACTAACTTTAACATGA

ATCGGAGGACAGCCCGTCGAACATCCATATGTCATCATTGGCCAA CTAGCAT
ATTGGAGGACAACCTGT TGAACACCCATATGTTATCATTGGACAAATCGCAT

CAATTCTTTATTT TTCTATTATCATTATCCTAATACCACTTATTAGCCTGATAG
CCATTCTATACTTCTCTATCATCATTATATTCATACCCCTAGCCAGCCTTGCAG

AGAACCACCTACTAAAATGAAGA
AAAACCACTTGTTAAAA TGAAGA

Plate 2.1. Aligned reference sequences of *M. brandtii* and *M. mystacinus*. Top sequence= *M. brandtii* reference sequence, bottom sequence= *M. mystacinus* reference sequence. (1-1139 bp., red= SNPs)

GATGAAATTTCGGATCTCTTTTAGGAATCTGCCTAATACTACAAATTCTGACA
GATGAAATTTCGGATCTCTTTTAGGAATCTGCCTNATACTACAAATTCTGACA

GGACTATTCCTAGCTATACACTATACGTCAGATACTGCAACAGCCTTCAACTC
GGACTATTCCTAGCTATACACTATACGTCAGATACTGCAACAGCCTTCAACTC

TGTTACCCATATCTGCCGAGACGTAAATTACGGCTGAGTCCTACGCTACTTAC
TGTTACCCATATCTGCCGAGACGTAAATTACGGCTGAGTCCTACGCTACTTAC

ATGCAAACGGAGCTTCTATATTTTTCATTTGCCTATATCTTCATGTAGGACGA
ATGCAAACGGAGCTTCTATGTTTTTCATTTGCCTATATCTTCATGTAGGACGA

GGACTTTACTACGGATCCTACATATATACAGAAACTTGAAATATTGGAGTAA
GGACTTTACTACGGATCCTACATATATACAGAAACTTGAAATATTGGAGTAA

TCTTACTATTTGCTGTAATAGCAACAGCTTTCATAGGATACGTACTCCCATGG
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GGCCAAATGTCCTTTTGAGGTGCAACCGTGATCACTAACCTACTCTCTGCCAT
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TCCATATATTGGTACAGACCTGGTAGAATGGATCTGAGGAGGCTTCTCCGTTG
TCCATATATTGGTACAGACCTGGTAGAATGGATCTGAGGAGGCTTCTCCGTTG

ACAAGGCCACCTTAACTCGATTCTTTGCTTTCCACTTTCTACTCCCATTTATCA
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TCTCCGCCATGGTCATAGTCCATCTTCTATTCCTTCACGAAACAGGATCTAAT

AACCCAACAGGAATTCCTTCTAATATAGACATAATTCCCTTTCACCCGTATTA
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TACAATTAAAGATATTTTGGGCCTACTACTAATAATCACGGCCCTTCTAGTAC
TACAATTAAAGATATTTTGGGCCTACTACTAATAATCACGGCCCTTCTAGTAC

TAGTACTATTTTCCCCTGACATACTAGGAGACCCCGATAACTATATACCAGCA
TAGTACTATTTTCCCCTGACATACTAGGAGACCCCGATAACTATATACCAGCA

AATCCTCTAAACACCCCTCCTCACATTAAACCAGAGTGATATTTTCTATTTGC
AATCCTCTAAACACCCCTCCTCACATTAAACCAGAGTGATATTTTCTATTTGC

ATACGCAATCCTACGATCAATTCCAAATAAATTAGGAGGAGTACTAGCTCTA
ATACGCAATCCTACGATCAATTCCAAATAAATTAGGAGGAGTACTAGCTCTA

GTCCTCTCTATCCTTATTCTAATTATTATCCCCC
GTCCTCTCTATCCTTATCCTAATTATTATCCCCC

Plate 2.2. Aligned sequences of *M.mystacinus*. Top sequence= reference sequence, bottom sequence= sequence 78 F. (828 bp. 52- 880) , yellow= N, red= SNP)

ACCACAACAGCTTTTAACTCTGTCACCCATATTTGCCGAGATGTAAACTATGG
ACCACAACAGCTTTTAACTCTGNCACCCATATTTGCCGAGATGTAAACTATGG

TTGAGTTCTACGCTACTTGCATGCAAATGGAGCCTCCATATTTTTTATCTGCCT
TTGAGTTCTACGCTACTTGCATGCAAATGGAGCCTCCATATTTTTTATCTGCCT

ATATCTCCATGTAGGACGGGGCCTTTACTATGGGTCCTATATATATACAGAAA
ATATCTCCATGTAGGACGGGGCCTTTACTNTGGGTCCTATATATATACAGAAA

CCTGAAATATCGGAGTTATTCTATTATTTGCTGTAATAGCAACAGCCTTTATA
CCTGAAATATCGGAGNTATTCTATTATTTGCTGTAATAGCAACAGCCTTTATA

GGATATGTACTTCCATGAGGACAAATGTCTTTCTGAGGAGCAACAGTAATTA
GGATATGTACTTCCATGAGGACAAATGTCTTTCTGAGGAGCAACAGNAATTA

CCAACCTGCTCTCTGCAATTCCGTACATTGGAACAGACCTTGTAGAATGAATC
CCAACCTGCTCTCTGCAATTCCGNACATTGGAACAGACCTTGTAGAATGAATC

TGAGGCGGCTTCTCTGTTGACAAAGCTACTTTGACCCGATTCTTTGCCTTTCA
TGAGGCGGCTTCTCTGTTGACAAAGCTACTTTGACCCGATTCTTTGCCTTTCA

CTTTTTACTCCCATTTATTATTGCAGCCATAGTCATAGTCCACCTCCTATTTCT
CTTTTTACTCCCATTTATTATTGCAGCCATAGTCATAGTCCACCTCCTATTTCT

TCACGAAACCGGATCCAATAACCCAACAGGAATCCCCTCCAACGCTGATATA
TCACGAAACCGGATCCAATAACCCAACAGGAATCCCCTCCAACGCTGATATA

ATCCCCTTCCACCCCTACTATACAATTAAAGACATTCTTGGCCTGCTATTAAT
ATCCCCTTCCACCCCTACTATACAATTAAAGACATTCTTGGCCTGCTATTAAT

AATTACAGTCCTACTCATACTAGTACTATTCTCCCCCGACCTGCTAGGAGACC
AATTACAGTCCTACTCATACTAGNACTATTCTCCCCCGACCTGCTAGGAGACC

CTGACAACTACACACCAGCGAACCCACTAAACACCCCTCCCCATATCAAACC
CTGACAACTACACACCAGCGAACCCACTAAACACCCCTCCCCATATCAAACC

GGAATGGTACTTTTTATTCGCATATGCAATTCTACGATCAATTCCAAACAAAC
GGAANGGNACTTTTTATTCGCATATGCAATTCTACGATCAATTCCAAACAAAC

TAGGAGGAGTGTTAGCCCTAGTACTATCAATCCTTATTCTAATTATCATTCCC
TAGGAGGAGTGNTNGCCCTAGGACTATCAATCCTTATTCTAATTATCATTCCC

CTACTCCACACCTCCAAACAACG
CTACTCCACACCTCCAAACAACG

Plate 2.3. Aligned sequences of *M. brandtii*. Top sequence= reference sequence, bottom sequence= sequence 109F. (764 bp. 175- 939 bp, yellow= N, no SNPs)

Feature	Minimum	Maximum	Mean	S.D.
Forearm length, mm	32.9	35.1	33.8	0.7
Tragus width, mm	1.2	2.0	1.8	0.2
Tragus length, mm	5.0	8.4	6.6	0.9
Thumb length, mm	4.0	6.3	5.3	0.5
Thumb claw length, mm	1.2	2.1	1.6	0.3
5^{th} digit length, mm	38.3	41.2	40.0	1.0
Calcar length, mm	10.2	16.6	13.0	2.2
Foot length, mm	5.0	8.2	7.3	0.8
Foot claw length, mm	1.0	2.3	1.7	0.3
Body mass, g	4.0	6.5	5.1	0.6

Table 2.3. *M. mystacinus* continuous variables (n= 16, females= 6, males= 10)

Feature	Minimum	Maximum	Mean	S.D.
Forearm length, mm	33.4	36.0	34.8	0.9
Tragus width, mm	1.6	2.4	2.0	0.3
Tragus length, mm	5.8	7.8	6.7	0.6
Thumb length, mm	5.5	6.4	5.9	0.3
Thumb claw length, mm	1.5	2.3	1.9	0.2
5^{th} digit length, mm	39.6	42.6	41.0	0.7
Calcar length, mm	10.2	18.2	15.4	1.8
Foot length, mm	6.8	8.3	7.7	0.5
Foot claw length, mm	1.7	2.5	1.9	0.2
Body mass, g	4.0	8.0	5.7	1.0

Table 2.4. *M. brandtii* continuous variables (n= 17, females= 4, males= 13)

Variable	*P*	Sign.	Sign. after multiple comparisons
Body mass	0.043	*	ns
Foot length	0.076	ns	ns
Thumb length	<0.001	***	***
Thumb claw length	0.001	***	***
Forearm length	0.010	**	**
Tragus length	0.214	ns	ns
Foot claw length	<0.001	***	***
Tragus width	0.077	ns	ns
5th digit length	0.027	*	ns
Calcar length	0.088	ns	ns

Table 2.5. Statistical results for Mann- Whitney U test on categorical morphological variables for *M. mystacinus* and *M. brandtii* on males only (n= 23, 10 and 13 respectively)

Variable	X^2	*P*	d.f.	Sign.	Sign. after multiple comparisons
Penis shape	13.917	<0.001	1	***	***
Tragus shape	10.828	<0.001	1	***	***
Lower jaw dentition	14.468	<0.001	2	***	***
Upper jaw dentition	23.241	<0.001	2	***	***
Ventral pelage colour	1.351	0.75<*P*<0.50	2	ns	ns
Dorsal pelage colour	1.173	0.50<*P*<0.25	2	ns	ns
Penis colour	5.750	0.75<*P*<0.50	1	ns	ns
Ear colour	8.250	0.50<*P*<0.25	1	ns	ns
Wing colour	6.305	0.05<*P*<0.25	2	ns	ns
Face colour	7.909	0.025<*P*<0.01	2	**	**

Table 2.6 Statistical results for Chi Square test on morphological categorical variables for *M. mystacinus* and *M. brandtii* on males only (n= 23, 10 and 13 respectively)

Variable	n	%	n	%	n	%
Penis shape	thin= 10	100				
Penis colour	pink= 3	30	lightpink= 3	30	pinkbeigetip= 4	40
Dorsal colour	golden= 11	68.75	darkbrown= 3	18.75	mediumbrown= 2	12.5
Ear colour	darkbrown= 7	43.75	mediumbrown= 9	56.25		
Face colour	darkbrown= 14	87.5	mediumbrown= 2	12.5		
Wing colour	darkbrown= 7	43.75	mediumbrown= 5	31.25	golden= 4	25
Ventral colour	lightgreybrown= 10	62.5	mediumgrey= 4	25	lightgoldenbrown= 2	12.5
Lower jaw dentition	small= 12	75	medium= 2	12.5	large= 2	12.5
Upper jaw dentition	none= 8	37.5	small= 6	56.25	large= 1	
Tragus shape	whiskered= 13	81.25	Brandt's= 3	18.75		

Table 2.7. *M. mystacinus* categorical variables (n= 16, females= 6, males= 10)

Variable	n	%	n	%	n	%	n	%	n	%
Penis shape	thin= 4	30.8	clubshaped= 9	69.2						
Penis colour	pink= 9	69.2	pinkbeigetip= 4	30.8						
Dorsal colour	golden= 9	52.9	darkbrown= 6	35.3	mediumbr. = 2	11.8				
Ear colour	darkbr.= 5	29.4	mediumbr.= 12	70.6						
Face colour	darkbr.= 7	41.2	mediumbr.= 8	47	golden= 2	11.8				
Wing colour	darkbr.= 6	35.3	mediumbr.= 11	64.7						
Ventral colour	lightgreybr.= 8	47	mediumgrey= 2	11.8	lightgoldenbr.= 3	17.6	lightbr.= 3	17.6	goldenbr = 1	5.9
Lower jaw dentition	small= 2	11.8	medium= 3	17.7	large= 12	70.5				
Upper jaw dentition	small= 2	11.8	medium= 7	41.2	large= 8	47				
Tragus shape	whiskered= 2	11.8	Brandt's= 15	88.2						

Table 2.8. *M. brandtii* categorical variables (n= 17, females= 4, males= 13), br.= brown

Variable	X^2	d.f.	*P*
Thumb length	13.676	1	<0.001
Thumb claw length	12.489	1	<0.001
5th finger length	7.083	1	0.008
Calcar length	4.509	1	0.034
Forearm length	7.512	1	0.006
Footclaw length	9.414	1	0.002
Face colour both spp.	3.235	2	0.198
Face colour *M.m*	2.718	1	0.099
Face colour *M.b*	0.910	1	0.340
Wing colour both spp.	1.437	2	0.488
Wing colour *M.m*	0.006	1	0.940
Wing colour *M.b*	0.306	1	0.580
Lower jaw dentition both spp.	10.894	2	0.004
Lower jaw dentition *M.m.*	9.673	1	0.002
Lower jaw dentition *M.b.*	8.069	1	0.005
Upper jaw dentition both spp.	16.121	3	0.001
Upper jaw dentition *M.m.*	5.247	1	0.022
Upper jaw dentition *M.b.*	3.489	1	0.062
Tragus shape *M.m.*	6.295	1	0.012
Tragus shape *M.b.*	12.407	1	<0.001
Overall	22.087	16	0.140

Table 2.9. Statistical results for logistic regression model on morphological variables for *M. mystacinus* (*M. m.*) and *M. brandtii* (*M. b.)* on males only (n= 23, 10 and 13 respectively)

Variable	X^2	d.f.	*P*	% corr. *M.m.*	% corr. *M. b.*	% corr. overall
Thumb claw length	16.380	1	<0.001	90	92	91
Lower jaw dentition	12.232	2	0.002	70	92	81
Upper jaw dentition	20.344	3	<0.001	90	92	91
Tragus shape	13.730	1	<0.001	80	84	82
Penis shape	14.741	1	<0.001	100	69	83

Table 2.10. Statistical results for logistic regression model on separate morphological variables of *M. mystacinus* (*M. m.*) and *M. brandtii* (*M. b.)* on males only (n= 23, 10 and 13 respectively)

Variable	Males n=10		Females n=6	
	mean	S.D.	mean	S.D.
Forearm length, mm	34.46	2.75	34.13	0.84
Body mass, g	4.95	0.44	5.33	0.88
Hand wing area, SHW, cm^2	13.63	1.25	14.02	1.89
Arm wing area, SAW, cm^2	19.65	2.28	20.51	2.37
Wing area, S, cm^2	81.64	3.96	86.18	6.55
Wing span, B, cm	22.68	0.51	22.87	1.25
Length of hand wing, LHW, mm	52.60	1.96	52.83	2.93
Length of arm wing, LAW, mm	47.10	4.12	48.50	3.21
Aspect ratio, A	5.89	0.51	6.16	1.47
Tip area ratio, TAR	0.71	0.11	0.70	0.14
Tip length ratio, TLR	1.13	0.12	1.08	0.10
Tip shape index, I	1.77	0.37	1.80	0.57
Wing loading, Mg/S, Nm^{-2}	6.40	0.24	6.08	0.50

Table 2.11. Flight morphology of *M. mystacinus* using conventions of Norberg and Rayner (1987)

Variable	Males n=13		Females n=4	
	mean	S.D.	mean	S.D.
Forearm length, mm	34.70	0.88	35.05	0.87
Body mass, g	5.46	0.85	5.25	1.66
Hand wing area, SHW, cm^2	14.09	1.27	13.97	1.36
Arm wing area, SAW, cm^2	20.29	1.90	21.04	2.53
Wing area, S, cm2	83.66	6.60	85.57	8.18
Wing span, B, cm	22.80	0.84	23.00	0.86
Length of hand wing, LHW, mm	52.85	2.30	51.75	2.50
Length of arm wing, LAW, mm	49.00	3.32	48.00	2.94
Aspect ratio, A	6.26	0.79	6.20	0.25
Tip area ratio, TAR	0.70	0.08	0.67	0.10
Tip length ratio, TLR	1.08	0.09	1.08	0.11
Tip shape index, I	1.94	0.55	1.61	0.20
Wing loading, Mg/S, Nm^{-2}	6.49	1.06	6.12	2.12

Table 2.12. Flight morphology of *M. brandtii* using conventions of Norberg and Rayner (1987)

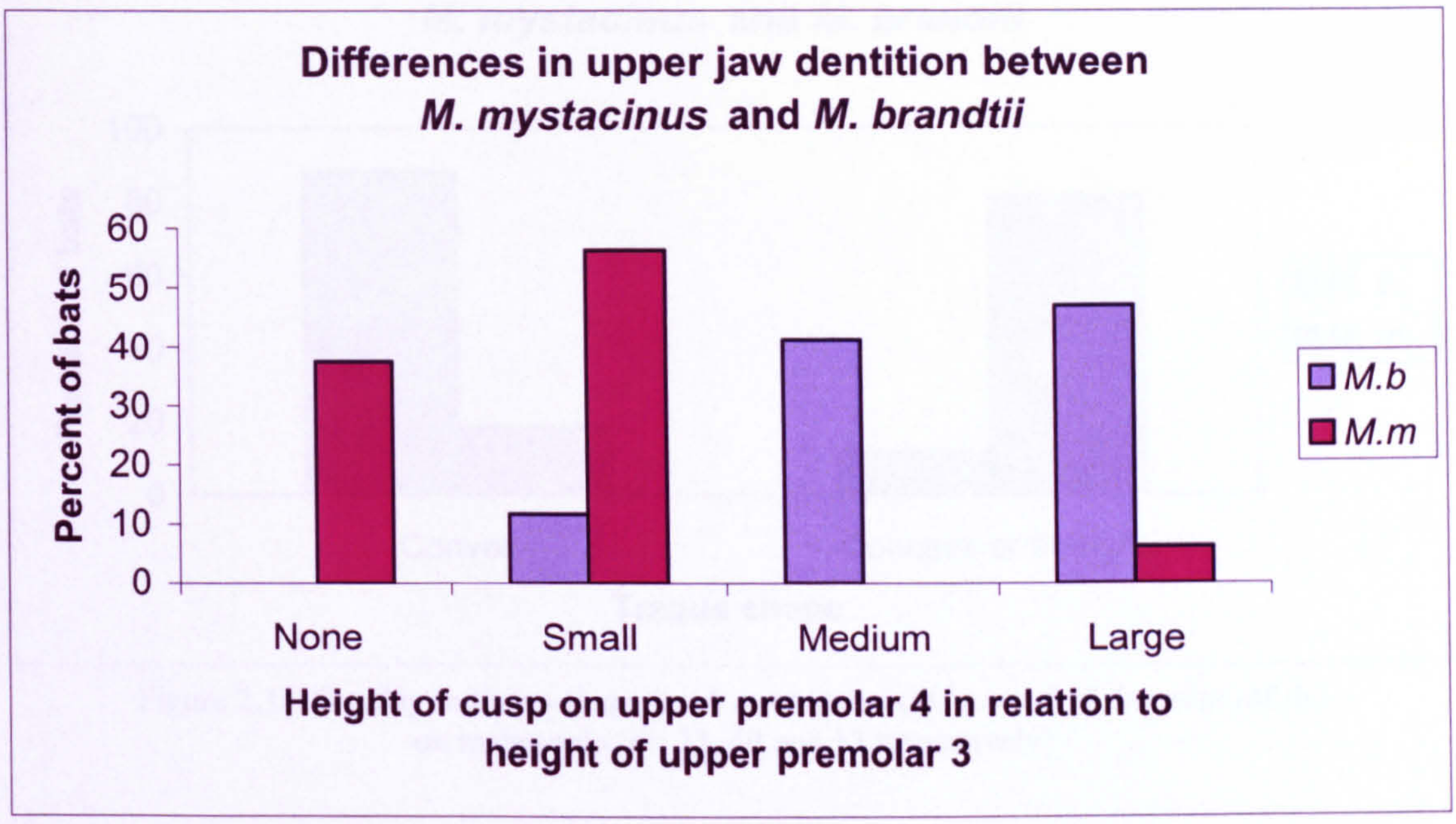

Figure 2.1a. Overlap in upper jaw dentition in *M. mystacinus* (*M. m.*) and *M. brandtii (M. b.)* on males only (n= 23, 10 and 13 respectively)

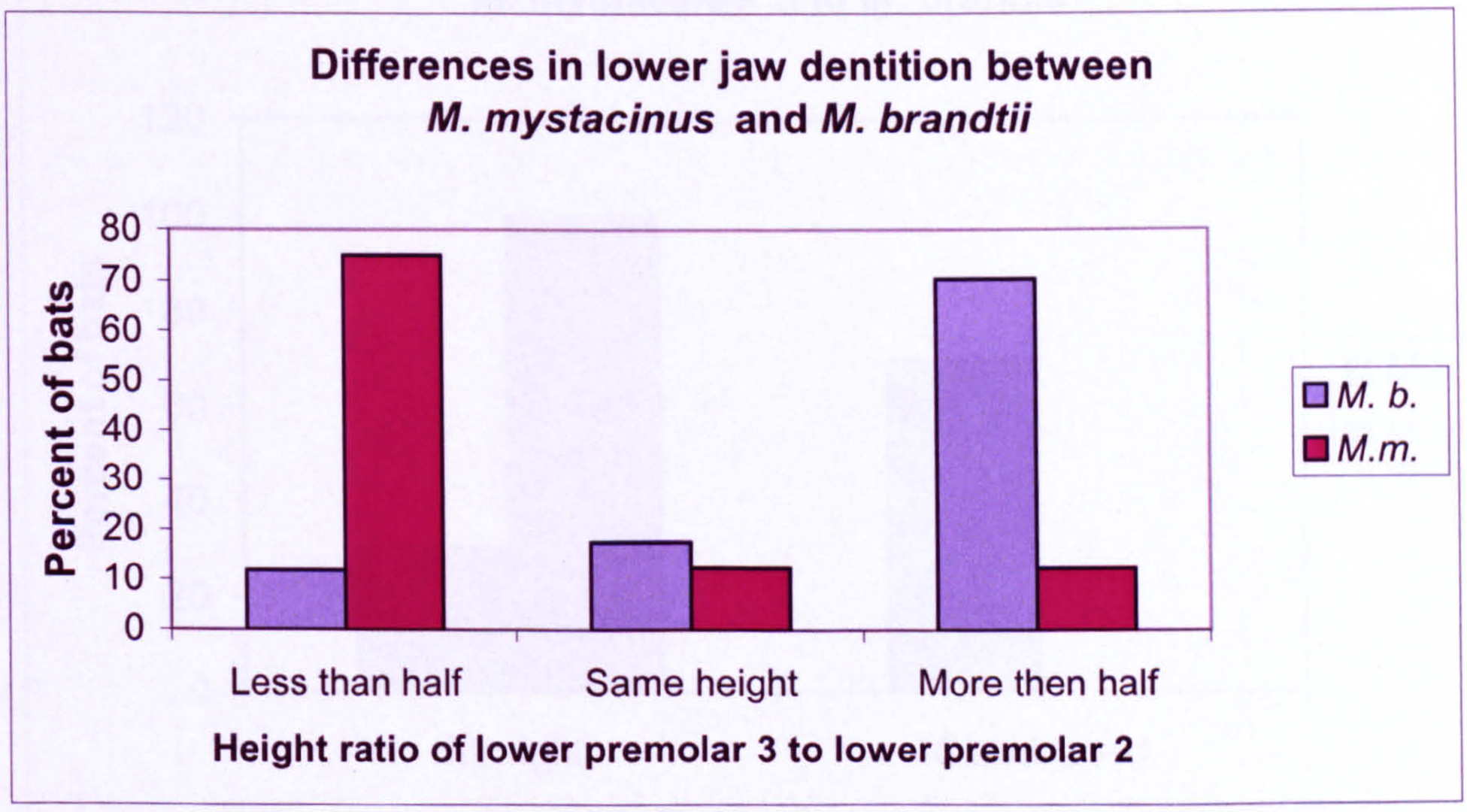

Figure 2.1b. Overlap in lower jaw dentition in *M. mystacinus* (*M. m.*) and *M. brandtii (M. b.)* on males only (n= 23, 10 and 13 respectively)

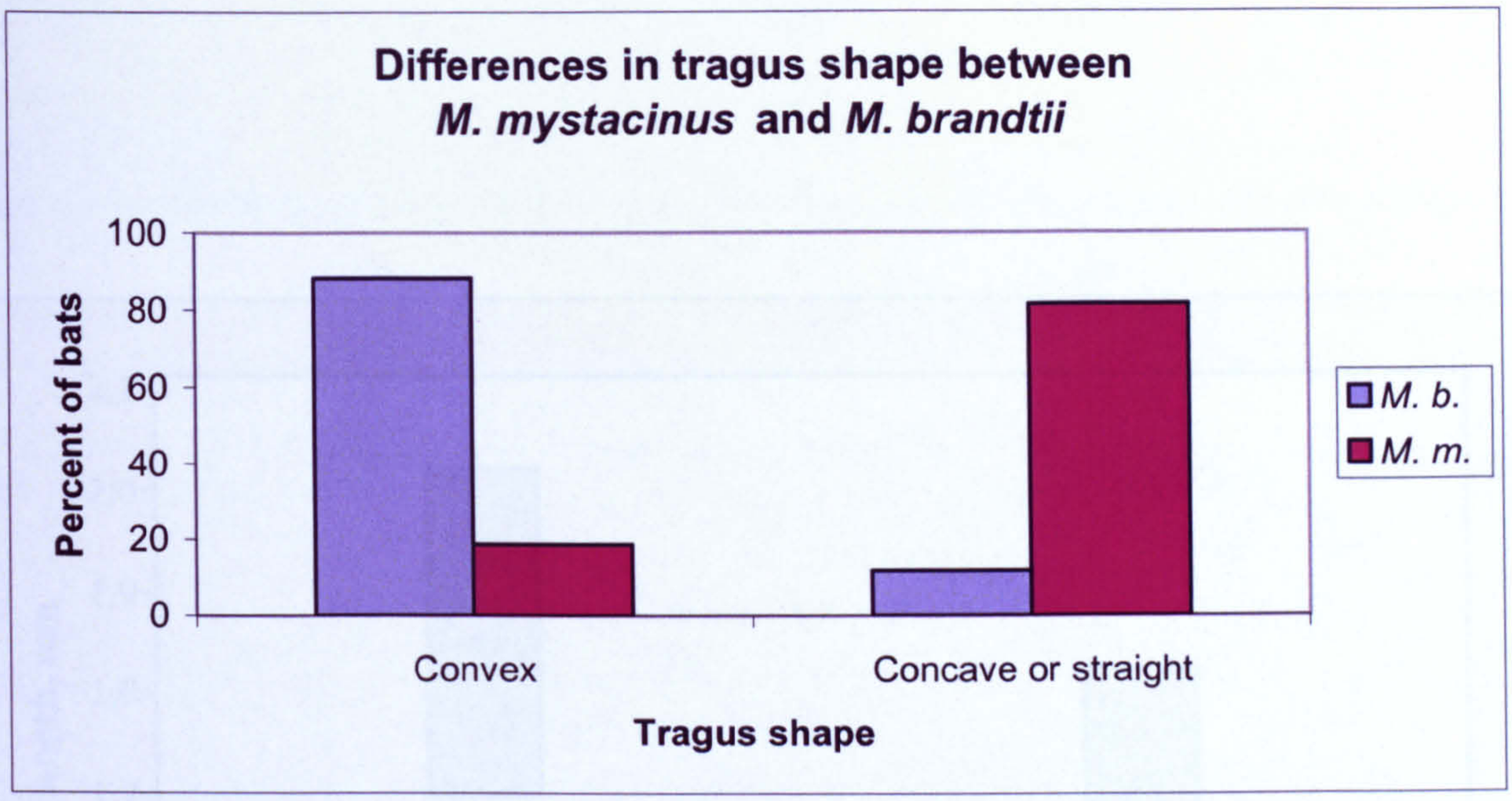

Figure 2.1c. Overlap in tragus shape in *M. mystacinus* (*M. m.*) and *M. brandtii (M. b.)* on males only (n= 23, 10 and 13 respectively)

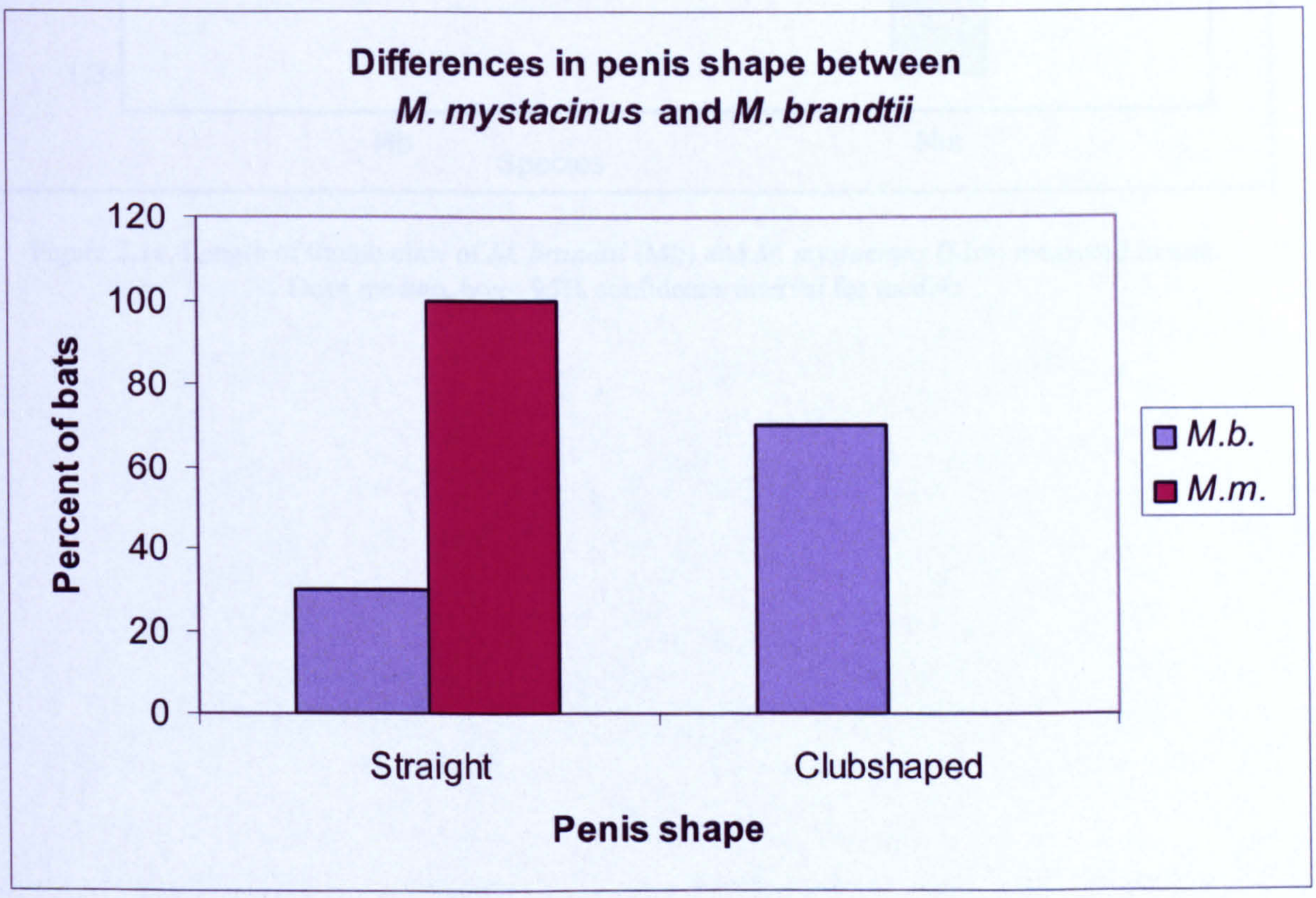

Figure 2.1d. Overlap in penis shape in *M. mystacinus* (*M. m.*) and *M. brandtii (M. b.)* on males only (n= 23, 10 and 13 respectively)

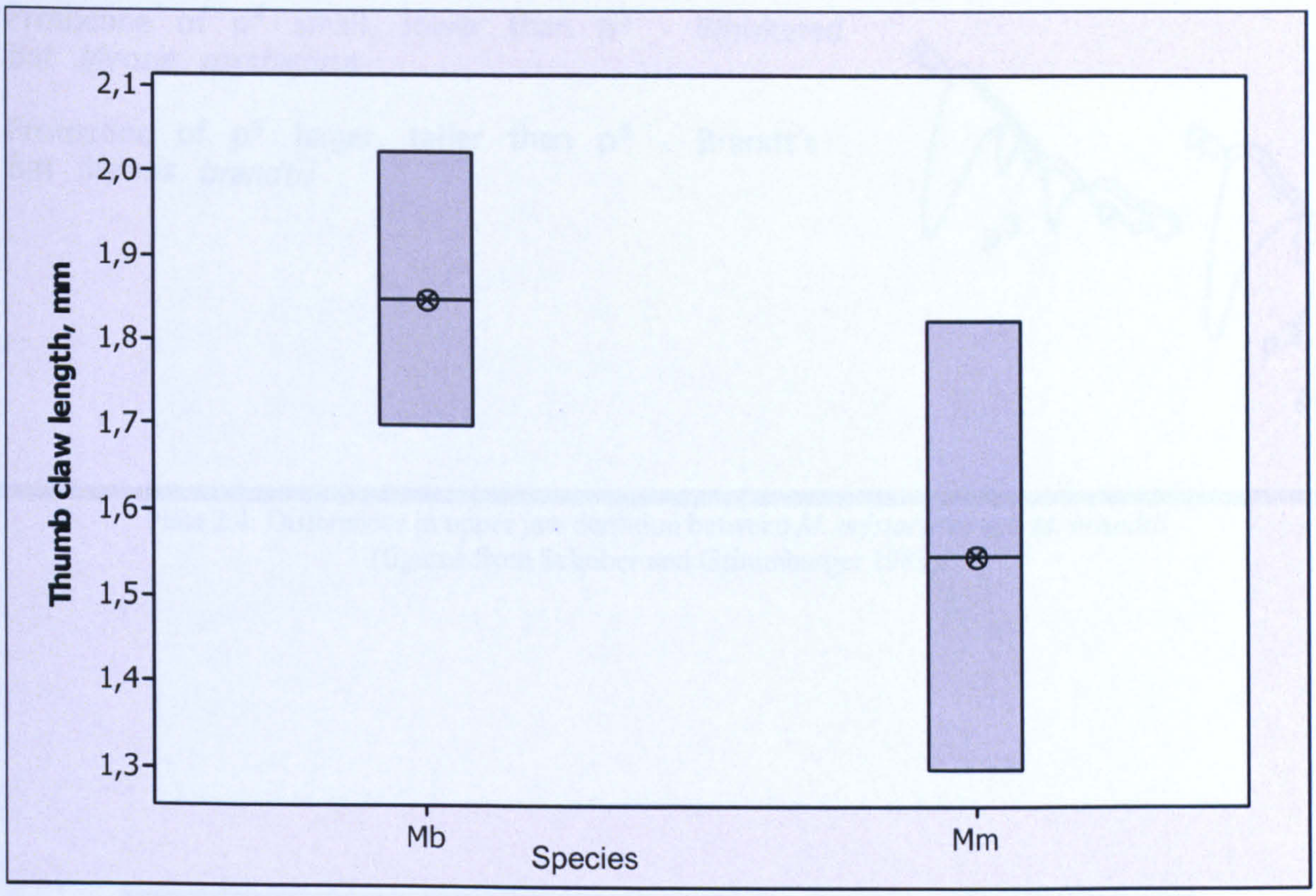

Figure 2.1e. Length of thumb claw of *M. brandtii* (Mb) and *M. mystacinus* (Mm) measured in mm.
Dot= median, box= 95% confidence interval for median

Protocone of p^4 small, lower than p^3 - Whiskered Bat *Myotis mystacinus*

Protocone of p^4 larger, taller than p^3 - Brandt's Bat *Myotis brandtii*

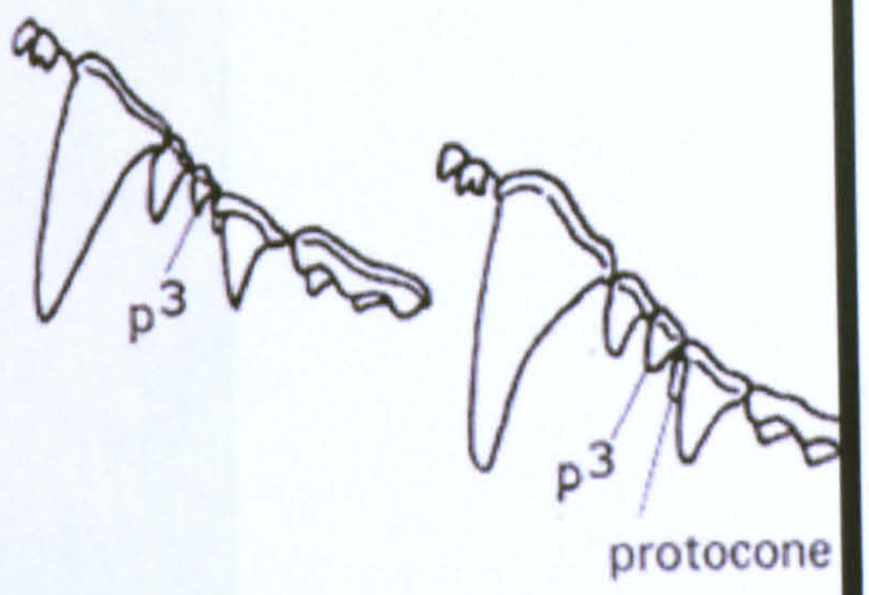

Plate 2.4. Differences in upper jaw dentition between *M. mystacinus* and *M. brandtii* (figures from Schober and Grimmberger 1989)

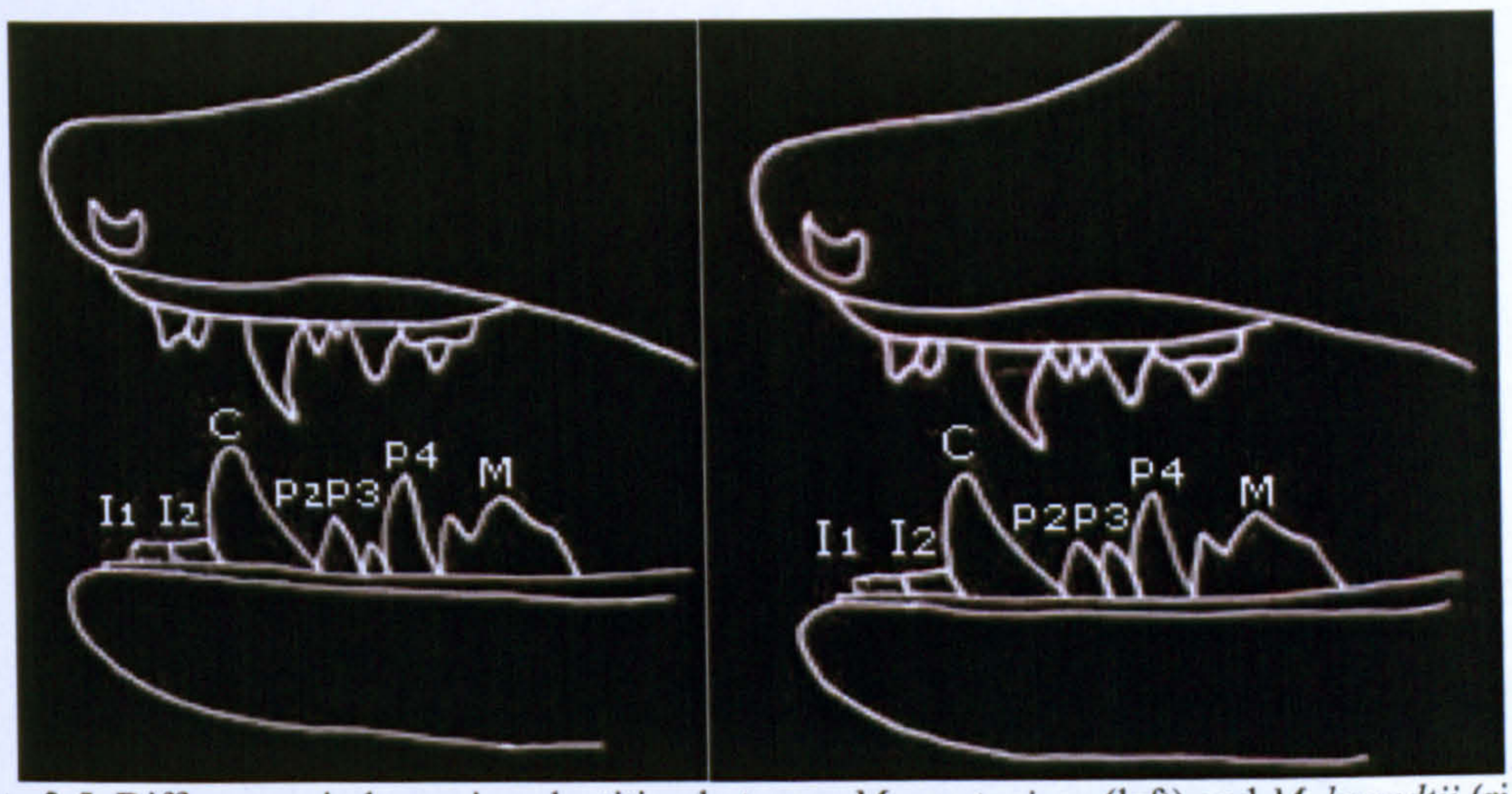

Plate 2.5. Differences in lower jaw dentition between *M. mystacinus* (left) and *M. brandtii* (right)

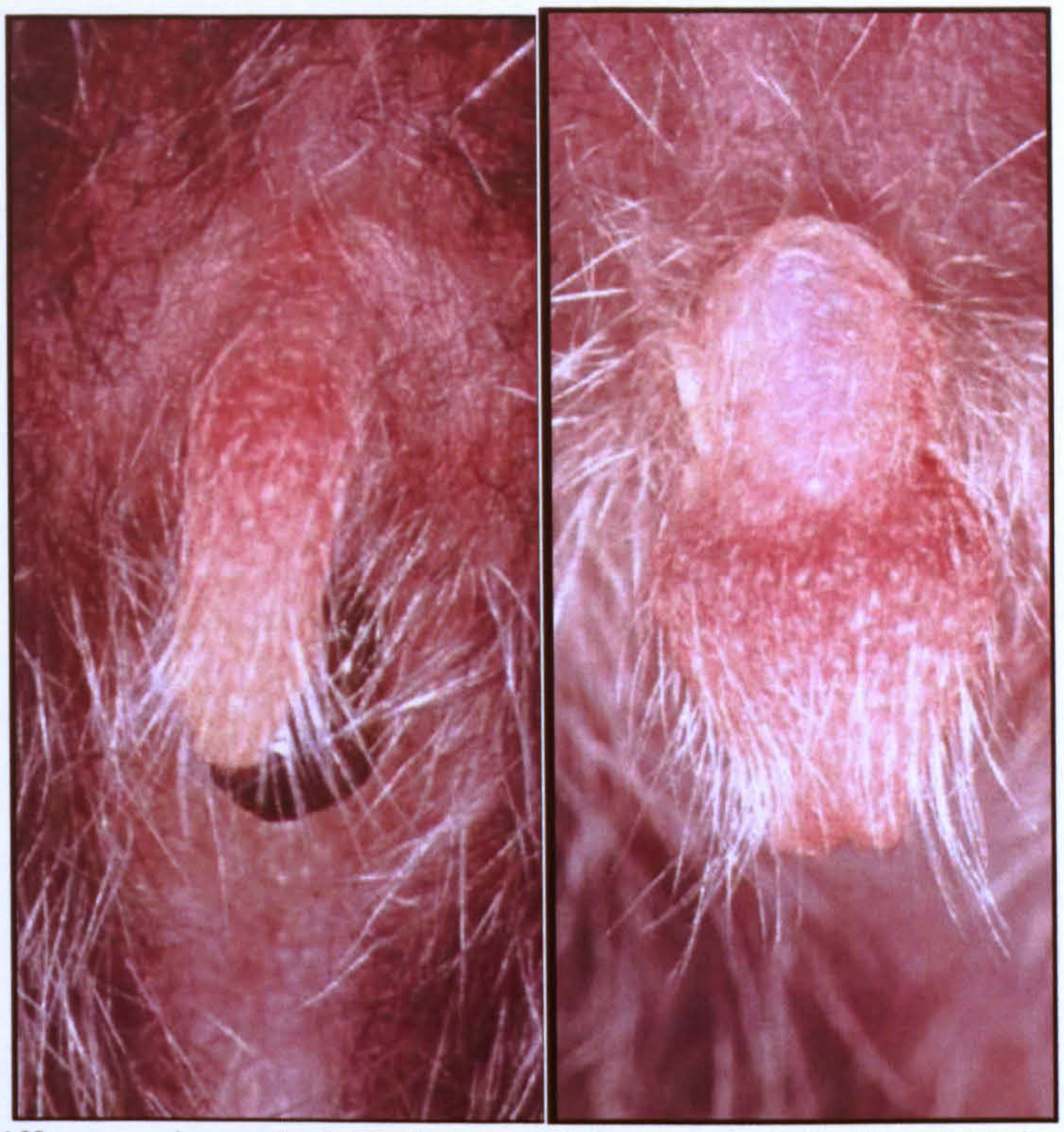

Plate 2.6. Differences in penis shape between *M. mystacinus* (left) and *M. brandtii* (right) (photos from Dietz and von Helversen 2004)

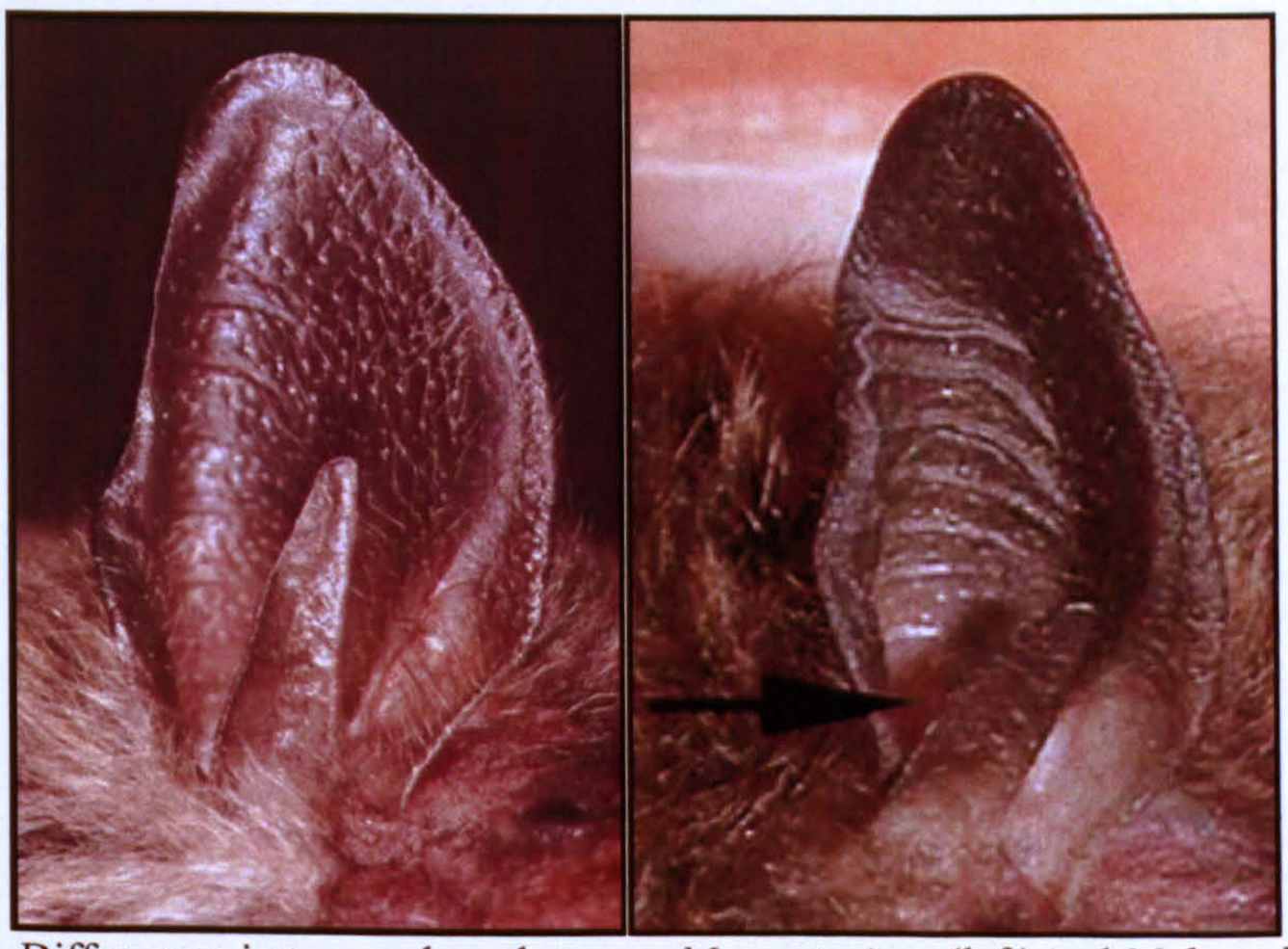

Plate 2.7. Differences in tragus shape between *M. mystacinus* (left) and *M. brandtii* (right)

3. HABITAT USE

ABSTRACT

Cryptic bat species have often been found to have different ecologies and consequently need different management strategies for conservation. However, previous studies have only researched cryptic bat species that are closely related. The cryptic species *M. mystacinus* and *M. brandtii* have different evolutionary histories and I therefore wanted to look into how they partition their resources. Habitat use is an important component of a species' ecological niche and I investigated this by radiotracking 12 *M. mystacinus* and 12 *M. brandtii*, using compositional analysis to investigate habitat selection.

M. mystacinus selected grassland (often grazed pasture surrounded by hedgerows) over all other habitat types, whereas *M. brandtii* selected coniferous woodland. This is an interesting result since coniferous woodland is often viewed as inferior habitat for bats in the UK. Arable land and built up areas were the least selected habitats types for the two species.

Although morphologically very similar, my study shows that the cryptic species have very different habitat use. The results suggest that differences in habitat preferences can occur between bat species that are almost identical in morphology. It is consequently important to take into consideration that morphology may be a weak indication of any ecological differences when managing these and other cryptic bat species for conservation.

3.1. INTRODUCTION

Long-term studies of the greater horseshoe bat (*Rhinolophus ferrumequinum*) discovered that good quality habitat around maternity roosts could be critical to their conservation and it was therefore recommended that key foraging habitats should be maintained and improved (Duvergè and Jones 1994). Such recommendations were fundamental in developing mechanisms of landscape improvement around maternity roosts funded through Countryside Stewardship Schemes. The management changes showed evidence of aiding a population increase of this species in Devon (Longley 2003). It was also suggested by Oakeley and Jones (1998) that the protection of prime foraging habitat around roosts may be vital. In order for appropriate conservation measures to be carried out for a particular bat species, knowledge of its prime foraging habitat is paramount.

Cryptic species are defined as species which are morphologically similar, but do not interbreed (Allaby 1996). Recently, a number of studies have focused on the ecology of cryptic species of bats (e.g. Arlettaz et al. 1993, Barlow and Jones 1997, Nicholls and Racey 2006b), because they provide us with an excellent tool to study community ecology. From a conservation point of view, studies of the ecology of cryptic species are extremely important because previous studies have often found clear differences in the diet and habitat use of cryptic species (e.g. Vaughan et al. 1997, Arlettaz 1999, Nicholls and Racey 2006b). However, these studies have only looked at cryptic species, which are closely related. A recent genetic study found that unlike the other study animals, the cryptic species *Myotis brandtii* and *M .mystacinus* have different evolutionary histories (Ruedi and Mayer 2001). They may therefore show even greater ecological differences than the cryptic bat species previously studied and need different management plans.

There is currently only one habitat use study focusing on both *M. mystacinus* and *M. brandtii*. This study was carried out in Germany using a methodology of surveying habitats around maternity colonies. Because bats actively select habitats from those available, even within their home ranges, measures of habitat around roosts may not necessarily identify the key habitats selected by bats however. The study concluded that *M. mystacinus* is most reliant on parks, gardens and villages, while *M. brandtii* select deciduous woodland, close to water (Taake 1984). Taake (1984) concluded that coniferous woodland, woodland edge and clearings were also important habitats for *M. brandtii*, but to a lesser degree. Note that there is

some disagreement in the literature on the importance of coniferous woodland for *M. brandtii* (Taake 1984, Ekman and DeJong 1996, Johansson and DeJong 1996). However, both species have wing morphologies and echolocation calls which indicate that they are edge or cluttered habitat foragers (Norberg and Rayner 1987).

The main aim of this study was to investigate habitat use and selection by *M. mystacinus* and *M. brandtii*, so that the conservation strategies for the species can incorporate management of appropriate feeding habitat.

3.2. METHODS

3.2.1. Study sites

The bats were caught at 2 maternity colonies for each species. The maternity colonies are all privately owned, Old-Stone houses located in south west England. Habitats surrounding the colonies are lowland landscapes consisting mostly of villages, woodlands, grasslands and arable fields. For locations and size of study colonies, refer to Table 3.1.

3.2.2. Choice of method

Broad- band acoustic surveys to determine habitat use could not be employed because of the similarities between the two species' echolocation calls. Light tagging could also not be employed due to the short lifespan of the tags and the great possibility of losing the tagged bats. Radiotracking however, has been used extensively to study movements, activity and foraging behaviour of bats (e.g. Tiedemann et al. 1985, Fleming and Heithaus 1986, Barclay 1989, Audet 1990, Jones and Morton 1992, Brigham 1993). Although there has been some debate on whether the transmitters influence the bats' behaviour significantly (Hickey 1990), the technique still remains powerful as a tool in determining the movements of an elusive and difficult-to-study group of animals (Jones and Morton 1992, Bontadina et al. 2002).

3.2.3. Capture and tagging of bats

Bats from one of the colonies (Stoford Manor, Taunton) were hand netted when exiting the roost. Bats from the remaining three colonies were hand netted inside the attic space because exit holes were either too difficult to reach, or bats emerged from a range of exits in an unpredictable manner.

Biometric data was obtained from all bats. Mass was recorded to an accuracy of 0.1 g by using a 30 g Pesola spring balance, forearm lengths were measured to the nearest 0.1 mm by using dial callipers. Animals were sexed and reproductive condition of females was assessed according to Anthony (1988) and Hutson and Racey (2004).

It is recommended that radio transmitters should add <5% of the mass of flying animals (Aldridge and Brigham 1988). However, a number of studies have reported minimal effect of radio transmitters on the foraging success of radiotagged bats, even with bats carrying transmitters weighing more than the recommended 5% of their body weight (e.g. Heithaus and Fleming 1978, Stebbings 1982, Adam et al. 1994, Entwistle 1994, Bontadina et al. 2002). Anderka and Angerhn (1992) suggested that some of the smaller species can carry transmitters exceeding the 5% limit. Gessaman and Nagy (1988) concluded that the increased flight cost resulted from drag induced by the transmitter rather than from the transmitter's mass. Additionally, Norberg and Rayner (1987) point out that small bats can carry larger loads relative to their body mass than large species, in addition to the mass-carrying capability possibly being greater in species with low wing loading, such as *M. mystacinus* and *M. brandtii*. The bats were equipped with 0.35 g radio transmitters (Micropip, Biotrack, Dorset, UK), which were on average 6.34% of the *M. mystacinus* bats' body mass and 6.00% of the *M. brandtii* bats' body mass. The possibility of the radio transmitters affecting the bats' flying behaviour is therefore low. In line with Bontadina et al. (2002), no heavily pregnant bats were selected for tagging and the lightest transmitters available were used.

Radiotagging followed standard procedure in that the transmitters were glued between the scapulae with a latex based glue (Skinbond, Smith and Nephew United Inc., Florida, USA) after the fur had been partially trimmed.

3.2.4. Radiotracking

The bats were radiotracked by car and on foot using a Lotek radio-receiver (Model STR_1000, Lotek Engineering, Ontario, Canada) and a 3 element Yagi aerial (Biotrack, Hants, UK). Radiotracking mostly followed the methodology of O'Donnell et al. (2001) and Bontadina et al. (2002). For each radiotracking session, 3 bats were tagged simultaneously. Each night one bat was tracked as the target bat, while fixes from the two other bats were taken if they could be picked up. Bats were followed from the night after the transmitter was attached and for varying lengths of time. Smith and Racey (2005a) report that a mean of 4.6 nights of foraging time is needed to reveal stable estimates of the habitat use for *M. nattereri*. When sampling for compositional analysis, it is appropriate to aim to obtain sufficient data from each animal to expect estimates of habitat use to be stable. However, animals with less data can also be included in the analysis. At the start of my project 3 bats were followed until the transmitters fell off, but this was reduced to three days with good data (i.e. contact with the bat for more than 90% of the time from emergence until sunrise).

Bats were monitored continuously from emergence until sunrise. Fixes were taken every 15 minutes and whenever a bat changed to a new location. The bats were extremely mobile and could cross their range in <15 minutes; therefore a 15 minute interval was considered appropriate for taking fixes, as suggested by O'Donnell (2001). All fixes were recorded as six figure grid references (British National Grid). Location of the bat was estimated using a combination of signal strength, knowledge of the terrain and observer experience. Fixes were calibrated with fixed transmitters placed at different locations in the study area, with radiotagged bats in known roosts and with free-flying bats in known locations. If the observer felt there was poor resolution for a fix (e.g. very faint signal or uncertain direction) the fix was omitted from the analysis. Fixes were taken mostly by using the homing in method (White and Garrot 1990), or simulated cross-triangulation by taking a bearing and then moving 50 metres in <30 seconds before taking a second bearing. However, this approach is only possible with reasonably accuracy when the animal is foraging in a small area for a reasonable length of time. Locations were assigned to accuracy classes 1-3 (50, 100, 150 metres) depending on the confidence in the estimated location. Problems associated with the accuracy of fixes are outlined in Kenward (1987). Direction of signal, signal strength and type of activity the bat was performing were recorded i.e. roosting, commuting, foraging, tree roosting.

3.2.5. Data analysis

The habitat types were identified by carrying out Habitat Phase 1 Surveys (JNCC 1993) and supplementing these with aerial photos (www.multimap.com) and 1: 25 000 Ordinance Survey Maps. Habitat Phase 1 data were also obtained from Somerset Environmental Records Centre for one of the study sites.

The Habitat Phase 1 Survey habitat types (JNCC 1993) were combined into 7 broad habitat types (Table 3.2.). Habitat maps were then created in the GIS software ArcView 3.1 (ESRI, UK) using digital maps downloaded from Digimap (www.edina.ac.uk/digimap/) and the software Map Manager 6.2 (ESRI, UK) to incorporate the files into ArcView. ArcView calculated habitat area totals for each study site and individual bats' minimum convex polygons (MCPs).

Comparisons of habitat types within each individual's home range were carried out using the Compositional Analysis Excel Tool 3.1. (P. Smith, University of Aberdeen) according to the methods of Aebischer et al. (1993). Available habitats used for the compositional analysis were the study areas (determined from the maximum foraging distance for each species, Chapter 4). This was chosen over MCPs (minimum convex polygons), which is the most commonly used definition of available habitat for compositional analysis (e.g. Davidson-Watts et al. 2006). Other studies on the habitat use of bats using compositional analysis have concluded that the results may depend on how available habitat is defined. Davidson- Watts et al. (2006) found little difference in results when using study area or MCPs as available habitat in a radiotracking study of *Pipistrellus pipistrellus* and *P. pygmaeus*. However, I got confusing results when running a compositional analysis using MCPs as available habitat. These results were not consistent with the observations made when radiotracking. A possible explanation for this result is the small size of MCPs (used habitat) compared to the 90% cluster polygons (available habitat, see below for further explanation). The analysis is therefore effectively an analysis of used vs. used habitat, rather than an analysis of available vs. used habitat. However, when a second compositional analysis was run with available habitat as study area (as discussed above), the results were consistent with observations made when radiotracking. Study area was therefore selected as a definition of available habitat rather than MCPs. The used habitats were defined as 90% cluster polygons (Kenward 1987). The radiotracked bats spent most of their time in relatively small areas, 90% cluster polygons

were therefore determined appropriate as a measure of used habitat because it reduces the chance of commuting and emergence increasing the size of the range disproportionately. Only the two main study colonies (Compton House, Bristol and Golden Mill, Truro) were used in the compositional analysis because sample sizes at the other colonies were too small, and bats were only followed to confirm that the behaviours at the main study colonies were not unusual. Note that mixed woodland and water had to be pulled out of the compositional analysis for *M. mystacinus* due to the difference between available and used habitat being too small and the size of these two habitat types in the study area also being too small. Default settings were used and 0 values were replaced with 0.001 for the compositional analysis, as suggested by Aebischer et al. (1993). All statistics, rank order and matrices are provided automatically by the Compositional Analysis Tool. Refer to Bontadina et al. (2002) and Russo et al. (2002) for the application of compositional analysis to bat radio-tracking.

3.3. RESULTS

3.3.1. Radiotracking

12 *M. mystacinus* and 13 *M. brandtii* were fitted with radiotransmitters during 2003 and 2004. 1 *M. brandtii* was not radiotracked due to tag failure and/or the bat moving away from the study area. Aebischer et al. (1993) report that 6 radiotagged animals constitute an absolute minimum for compositional analysis. 9 *M. mystacinus* were captured and radiotracked around the colony at Compton House (Bristol), the remaining 3 bats were captured and radiotracked around the colony at Stoford Manor (Taunton). 11 of the *M. brandtii* were captured and radiotracked around the colony at Golden Mill (Truro), the remaining 1 bat was captured and radiotracked around the colony at Church House (South Brent).

Only the bats radiotracked from the main study colonies were used in the compositional analysis. The 9 *M. mystacinus* from the main study colony were radiotracked for a total of 69 bat nights, with an average of 7.67 nights per bat and a total of 38 bat nights with good data (defined as being able to stay in contact with the bat for 90% of the night from emergence until sunrise), with an average of 4.22 nights with good data per bat. The radiotransmitters attached to the *M. mystacinus* had a life of 6- 14 days, with an average of 9.44 days, but this is difficult to estimate as some transmitters were still working when the radiotracking session

ended. The 11 *M. brandtii* from the main study colony were radiotracked for a total of 77 bat nights, with an average of 7 nights per bat and a total of 58 bat nights with good data and an average of 5 nights with good data per bat. The radiotransmitters had a life of 4-11 days, with an average of 8 days. The bats were radiotracked between May and August, i.e. during pregnancy and lactation. All the radiotracked bats were adult females, except one male *M. mystacinus* tagged at Compton House, Bristol. The two species were radiotagged on similar dates (U= 96.0, N1= 11, N2= 9, P= 0.1480). For further information on sex, colony and tracking dates of each bat refer to Table 3.3. For numbers of days tracked, number of days tracked with good data and tag life for each species refer to Table 3.4.

3.3.2. Habitat preferences

The overall composition of habitats available (i.e. the habitat composition of the study area) for *M. brandtii* (n= 11) over 3450 ha was 48.24% grassland, 34.26% arable land, 8.01% built up areas, 6.35% deciduous woodland, 2.01% water, 0.58% mixed woodland and 0.56% coniferous woodland (Figure 3.1). The overall composition of habitats available (i.e. the habitat composition of the study area) for *M. mystacinus* (n=9) over 2682 ha was 61.94% grassland, 28.97% built up areas, 6.78% deciduous woodland, 1.99% arable land, 0.12% coniferous woodland, 0.12% water and 0.06% mixed woodland (Figure 3.2). The study area for both species is dominated by grassland.

For *M. brandtii*, mean proportions of each habitat type within the study area and within 90% cluster polygons are shown in Figure 3.1. Percentage habitat composition of the study area (available) was significantly different from 90% cluster polygons (used) (weighted mean Wilk's Λ= 0.0509, X^2= 32.7478, d.f.= 6, P< 0.0001, randomisation P<0.003). A ranking matrix (Table 3.5) ordered the habitats in sequences from most to least used habitats as follows: coniferous woodland> mixed woodland> grassland> deciduous woodland> water> built up areas> arable land (where a habitat preceding a > symbol was preferred to that immediately following the symbol). Coniferous woodland was used over all other habitat types. Arable land, built up areas and water were the habitat types least selected by *M. brandtii*. *M. brandtii* therefore seem to be most reliant on woodland, especially coniferous woodland, however there was only significant differences between coniferous woodland and arable and built up areas. Grassland in the core foraging areas were under 10% semi-natural grassland and the rest being semi-improved or improved grassland. 92.3% of the grassland in the core foraging areas was surrounded by hedgerows and 7.69% was used as pasture grazed

by horses and cattle. It is possible that a larger percentage of the grassland was grazed during other times of the season.

For *M. mystacinus*, mean proportions of each habitat type within the study area and within 90% cluster polygons are shown in Figure 3.2. Percentage habitat composition of the study area (available) was significantly different from 90% cluster polygons (used) (weighted mean Wilk's $\Lambda = 0.0033$, $X^2 = 73.9595$, d.f.= 4, $P < 0.0001$, randomisation $P < 0.009$). A ranking matrix (Table 3.6.) ordered the habitats in sequences from most to least used habitats as follows: grassland>>> built up areas> deciduous woodland> coniferous woodland>arable land (where a habitat preceding a > symbol was used to that immediately following the symbol and where >>> denotes a significant difference between adjacent habitat types). Grassland was used significantly more often that all other habitat types, while arable land and coniferous woodland were the habitats least used by *M. mystacinus*. Grassland in the core foraging areas were under 10% semi-natural grassland, the remaining grassland was improved or semi-improved grassland. 90.91% of the grassland in the core foraging areas was surrounded by hedgerows and 58.33% was used as pasture, grazed by cattle or horses. It is possible that more of the grassland was used for grazing during other times of the season. For MCP (minimum convex polygon) and 90% cluster polygons for 1 *M. mystacinus* and 1. *M. brandtii* superimposed on a habitat map refer to Plate 3.2.

Note that the compositional analysis was only carried out for the bats from the main study colonies (i.e. Compton House, Bristol and Golden Mill, Truro). However, the remaining 3 *M. mystacinus* radiotracked at Stoford Manor (Taunton) and the 1 *M. brandtii* radiotracked at Church House (South Brent) showed similar habitat use as the bats from the main study colonies. The *M. mystacinus* radiotracked at the secondary study colony also had their main foraging sites in grassland (improved or semi-improved). 100% of the grassland in the core foraging areas was surrounded by hedgerows and at least 30% was used as pasture grazed by cattle during the radiotracking period. It is possible that the other main foraging sites were grazed at other times of the season. The 1 *M. brandtii* tracked at the secondary study colony had its core foraging areas in deciduous woodland.

3.4. DISCUSSION

3.4.1. Habitat use

On the island of Gotland in Sweden *M. brandtii* was found in woodland clearings and it was reported that the best foraging grounds for the species were grazed woodland (Ahlén 1994). Lehmann (1983-84) and Gerell (1987) found *M. brandtii* mostly in boreal forests of northern and eastern Europe. Further studies found all but one roost in the forest or on the forest edge, again suggesting that the bats are closely related to woodland (Strelkov 1983, Taake 1984). A German study by Taake (1984) found that *M. brandtii* show a close connection to wooded areas. The study also showed that the bats have a close connection to water, although this was not essential. Deciduous woodland with particularly damp areas close to water was found to be the principal habitat of this bat, but coniferous woodland, woodland edge and clearings were also frequently selected (Taake 1984). However, this is a study using habitat surveys around maternity colonies, not radiotracking. As the results from my study shows, bats select particular foraging habitats within their range. Note that there is some disagreement in the literature on the importance of coniferous woodland for the species (e.g. Taake 1984, Ekman and DeJong 1996, Johansson and DeJong 1996). The bats avoid open areas and use hedgerows and other linear features as flight paths between foraging grounds and roosts (Taake 1984). DeJong (1994), Ekman and DeJong (1996) and Johansson and DeJong (1996) found that the species is negatively affected by habitat isolation. This suggests that the species is particularly vulnerable to increased forest patchiness. Hypotheses from these studies failed to explain why the species avoids open habitats and as a consequence are negatively affected by isolation.

My study supports the findings from the published studies reported above in that *M. brandtii* is closely associated with woodland. However, coniferous woodland was selected over mixed or deciduous woodland and riparian habitats were not a preferred habitat type in my study. The coniferous woodland selected in my study was coniferous plantation woodland with little undergrowth dominated by Norway spruce *(Picea abies).* Coniferous woodland is often viewed as inferior habitat for foraging bats in the UK because most coniferous trees are non-native and often have low insect diversity associated with them (Stokoe and Stovin 1944, Freeeman 1945, Stokoe and Stovin 1948, Ford 1949, Walsh 1954, Southwood 2007). Moreover, coniferous trees are often removed for harvesting before they can develop cavities suitable for roosting bats. Nevertheless, native coniferous woodland can be important foraging

habitats for bats in Europe (e.g. Johansson and DeJong 1996), and the importance of coniferous woodland in the UK is being increasingly recognised, e.g. commercial coniferous plantations for *Myotis nattereri* in Scotland (Mortimer 2005). For a photo of the key foraging habitat of *M. brandtii*, refer to Plate 3.3.

These differences between studies may be due to differences in habitat types surrounding the study colonies, this is unlikely however, as the results for the secondary study site used in my study were similar to the results from the main study site, in that *M. brandtii* selects woodland. Differences between studies could also be due to my study colonies being at the limit of the species' geographical range or the fact that different methodologies were used in the studies (i.e. radiotracking in my study and acoustic or habitat surveys around maternity colonies in other published studies, methodologies with some inherent biases). Another possible explanation may be the species having extremely flexible foraging behaviour and changing their habitat use if the surrounding habitat is very different in composition or insect availability.

M. mystacinus show a distinctively weaker connection to wooded areas than *M. brandtii*. This species is most frequently found in open areas such as parks, gardens and villages according to a German survey of habitat types around maternity colonies. *M. mystacinus* is therefore considered a house-dwelling more than a forest-dwelling bat (Taake 1984). However, the bats also select woodland paths, woodland edges and woodland to a lesser extent (Taake 1984). The same study showed that although *M. mystacinus* has a preference for flowing water with riperian vegetation, it is not a necessary precondition for the occurrence of *M. mystacinus* in an area (Taake 1984).

Aldridge found significant correlations between the behavioural clutter index and the foraging zone utilisation index suggesting that different bat species select their foraging sites on the basis of their ability to fly in clutter. *M. mystacinus* has a foraging style index of 3 indicating that it forages in pastures. However, Buckley (2004) reported that *M. mystacinus* selected tree lines, deciduous woodland centre, mixed woodland edge and riparian habitats while coniferous woodland, intensive grassland and lake habitats were avoided. Deciduous woodland edge, mixed woodland centre and young plantation were used in proportion to their availability. Vaughan et al. (1997) found that *M. mystacinus* foraged mainly over lakes and to a lesser extent in semi-natural woodland and mixed plantation. Open areas in woodland (e.g.

clearings, paths and roads), parks, woodland edge and nutritious lakes were found to be habitat types preferred by *M. mystacinus* in studies by Ahlén and De Jong (1996) and Ahlén (1997). Clearings in woodland (especially deciduous and mixed) have also been found to be important in Holland (van der Coelen and Verheggen 1997). In the Czech Republic *M. mystacinus* are found in humid and woody areas ranging from medium to high altitudes (Kratky 1988). Johansson and DeJong (1996) concluded that the species is negatively affected by forest patchiness.

My findings correspond with the results from most of the published studies above in that *M. mystacinus* forage over different types of grassland. The grassland was mostly improved or semi-improved. Over 90% of the grassland in the core foraging areas was surrounded by hedgerows, about 60% was used as pasture grazed by cattle or horses at the time of radiotracking (although it is possible that more is grazed at other times of the year). Hedgerows tend to support large numbers of Diptera (Lewis 1969, Kirby 1992), one of the major components of the diet of *M. mystacinus* (Chapter 5). Verboom and Spoelstra (1999) found a significant relationship between bat (*Pipistrellus pipistrellus*) and insect abundances only when the tree-line bordered insect-rich grassland in a study in the Netherlands. Wind also only partly explained distances the bats flew from the tree lines. Acoustic landmarks and predator avoidance were therefore offered as alternative explanations for this behaviour. Similarly, e.g. Limpens et al. (1989), Hutson (1993) and Oakeley and Jones (1998) suggest that hedgerows are important as corridors for flying bats or for navigational purposes. The differences between studies, similarly to *M. brandtii*, may be due to differences in habitat types surrounding the study colonies, this is unlikely however as the *M. mystacinus* radiotracked at the secondary study site showed similar habitat use. The use of different methodologies (habitat surveys around maternity colonies and acoustic surveys in other studies, methods with inherent biases, and radiotracking in my study) may explain differences between studies. Additionally, my study colonies being at the limit of the species' geographical range may also lead to such differences. For a photo of the key foraging habitat of *M. mystacinus*, refer to Plate 3.4.

Nyholm (1965) made some very interesting discoveries during his study in Finland on *M. mystacinus*. He found that the bats changed their hunting ground as the nights became darker, towards the end of the summer. Both habitat type, size and number of individuals using the

area changed. Nyholm suggested that this might be linked to changes in lighting conditions and not changes in insect availability. Duvergé and Jones (1994) found similar behaviour in *R. ferrumequinum.* The bats switched their foraging grounds from woodland to pasture; this corresponded with a change in diet. Seasonal differences in habitat use could not be analysed in my study due to the small sample size and the short life or the radio tags.

Both species showed low levels of preference for arable habitats. Arable land is a habitat type rarely used by British bats (Vaughan et al. 1997), probably because insect densities have been reduced by agricultural intensification (Wickramasinghe et al. 2004). *M. brandtii* also showed low levels of preference for built up areas. This habitat type can have a number of negative impacts on bat foraging, including low insect abundance and diversity (Blair and Launer 1997). Grazed pasture and woodland, the prime foraging habitats of *M. mystacinus* and *M. brandtii* on the other hand, have the highest density and variety of insects (Service 1973, Stebbings 1982).

Habitat use studies of the cryptic *P. pipistrellus* and *P. pygmaeus* show that while *P. pygmaeus* foraged predominantly within woodland edge and riparian habitats, *P. pipistrellus* demonstrated a wider selection of habitats (Nicholls and Racey 2006b, Davidson-Watts et al. 2006). Interestingly however, most pairs of cryptic species for which habitat segregation has been established belong to the *Myotis* genus. Both *M. leibii* and *M. californicus*; *M. yumanensis* and *M. lucifugus* and *M. lucifugus* and *M. volans* segregated more or less through habitat use (Herd and Fenton 1983, Saunders and Barclay 1992). Arlettaz (1999) also found habitat use differences between the cryptic species *M. myotis* and *M. blythii*. While *M. myotis* select freshly cut meadows and forests without undergrowth, i.e. habitats with easy access to ground dwelling prey, *M. blythii* select grassland and unmown meadows.

3.4.2. Morphology as a predictor of habitat use

There is no clear link between any morphological differences between *M. mystacinus* and *M. brandtii* and differences in habitat use (Jones 1991, Chapter 2) and Parsons and Jones (2000) found only minor differences in the echolocation calls between the species. Norberg and Rayner (1987) describe *M. mystacinus* as bats with low/average wing loading, low aspect ratio and short rounded wing tips. From this information they predict that foraging by this bat would be by slow hawking close to or within clutter, as the wing designs make them highly manoeuvrable for insect hawking near vegetation. This type of foraging strategy for *M.*

mystacinus was also proposed by Aldridge (1985) from flight agility experiments on this bat. He suggested that *M. mystacinus* would not be able to utilise very cluttered situations or to catch aerial prey in the open, but would use the interface between these two strategies, hunting in moderately cluttered woodland edges, catching prey by flushing or gleaning.

Morphological similarities are often assumed to reflect similarities in habitat use. This does not correspond with the results from my study. *M. mystacinus* and *M. brandtii* are not closely related (Mayr and Ruedi 2001); their similarities in morphology are therefore probably due to convergent evolution. It is possible, that past competition (referred to as the "ghost of competition past") may have led to these differences in habitat use. Additionally, species on the western and northern edge of their geographical ranges such as *M. mystacinus* and *M. brandtii* may be expected to exhibit narrower ecological tolerance; therefore competition may limit their populations to a greater extent. This is further discussed in section 6.1

3.5. CONCLUSION

My study has been the first to compare the habitat use of *M. brandtii* and *M. mystacinus* using radiotracking. This method has the advantage that the breeding status and sex of the studied bats are known and is therefore extremely useful for investigating the habitat use of the bats in the most critical part of their lifecycle i.e. pregnancy and lactation, when energy demands are at their peak (Kurta et al. 1989). The findings of this study will contribute to developing the appropriate habitat management around maternity sites, which are of prime conservation concern because they are used for rearing young and contain large numbers of bats. However, studies determining the habitat use of a larger sample of bats from several study sites in different geographical areas should also be carried out. As radiotracking is an expensive and labour intensive method to study habitat use, studies may also be carried out as habitat surveys around maternity roost in a radius similar to the maximum foraging distance of *M. mystacinus* and *M. brandtii* (2.3 and 3.2 km respectively). Radiotracking *M. mystacinus* and *M. brandtii* simultaneously both in areas of sympatry and allopatry would also provide some interesting results.

Most importantly however, my study emphasises that morphological differences may be a poor indicator of possible ecological differences between bat taxa because great differences in

habitat use can occur between cryptic species that are almost identical in morphology and echolocation, but are not closely related.

Roost name	Nearest big town	County	Grid reference	Species	No. of adult bats
Compton House	Bristol	Bristol	ST568818	*M. mystacinus*	25+
Stoford Manor	Taunton	Devon	ST180219	*M. mystacinus*	58+
Church House	South Brent	Devon	ST692602	*M. brandtii*	51+
Golden Mill	Truro	Cornwall	SX928467	*M. brandtii*	61+

Table 3.1. Description of study colonies of *M. mystacinus* or *M. brandtii*

Habitat type	Description
Water	Standing water (lakes and ponds), running water (rivers, streams)
Arable	Arable land
Grassland	Improved, semi-improved, semi-natural and amenity grassland
Built up	Villages, farms, residential housing, roads, bare ground, industrial areas, airports
Deciduous woodland	Semi-natural broadleaf woodland, plantation broadleaf woodland, orchards
Coniferous woodland	10% or less broadleaf canopy in semi-natural or plantation coniferous woodland
Mixed woodland	10% or more of broadleaf or coniferous canopy in semi-natural or plantation woodland

Table 3.2. Description of habitat types

Bat id.	Species	Maternity colony	Sex	Tracking dates
288	*M. brandtii*	Golden Mill, Truro	Female	26.05- 01.06.04
335	*M. brandtii*	Golden Mill, Truro	Female	24.05- 31.05.04
233	*M. brandtii*	Golden Mill, Truro	Female	24.05- 01.06.04
269	*M. brandtii*	Golden Mill, Truro	Female	26.05- 28.05.04
301	*M. brandtii*	Golden Mill, Truro	Female	25.05- 29.05.04
227	*M. brandtii*	Golden Mill, Truro	Female	24.05- 02.06.04
248	*M. brandtii*	Golden Mill, Truro	Female	15.07- 18.07.04
261	*M. brandtii*	Golden Mill, Truro	Female	09.07- 18.07.04
279	*M. brandtii*	Golden Mill, Truro	Female	10.07- 18.07.04
305	*M. brandtii*	Golden Mill, Truro	Female	15.07- 18.07.04
339	*M. brandtii*	Golden Mill, Truro	Female	10.07- 18.07.04
312	*M. brandtii*	Church House, South Brent	Female	21.07- 23.07.04
334	*M. mystacinus*	Compton House, Bristol	Female	28.05- 04.06.03
214	*M. mystacinus*	Compton House, Bristol	Female	28.05- 02.06.03
280	*M. mystacinus*	Compton House, Bristol	Female	28.05- 06.06.03
314	*M. mystacinus*	Compton House, Bristol	Female	24.07- 05.08.04
229	*M. mystacinus*	Compton House, Bristol	Female	24.07- 03.08.04
267	*M. mystacinus*	Compton House, Bristol	Male	24.07- 03.08.04
293	*M. mystacinus*	Compton House, Bristol	Female	10.06- 15.06.04
330	*M. mystacinus*	Compton House, Bristol	Female	10.06- 15.06.04
235	*M. mystacinus*	Compton House, Bristol	Female	10.06- 14.06.04
262	*M. mystacinus*	Stoford Manor, Taunton	Female	07.08- 09.08.04
294	*M. mystacinus*	Stoford Manor, Taunton	Female	07.08- 09.08.04
310	*M. mystacinus*	Stoford Manor, Taunton	Female	07.08- 09.08.04

Table 3.3. Sex, colony and tracking dates of the radiotracked *M. mystacinus* and *M. brandtii*

Variable	Species	Range	Mean
Number of days tracked	*M. brandtii*	3.0- 10.0	7.0
	M. mystacinus	3.0- 10.0	7.67
Number of days with good data	*M. brandtii*	3.0- 10.0	5.0
	M. mystacinus	3.0- 7.0	4.22
Tag life, number of days	*M. brandtii*	4.0- 11.0+	8.0+
	M. mystacinus	6.0- 14.0+	9.44+

Table 3.4. Mean and range of number days tracked, number of days with good data and tag life for *M. mystacinus* and *M. brandtii* from the main study colonies
(with good data defined as staying in contact with the bat for 90% of the time from emergence to sunrise)

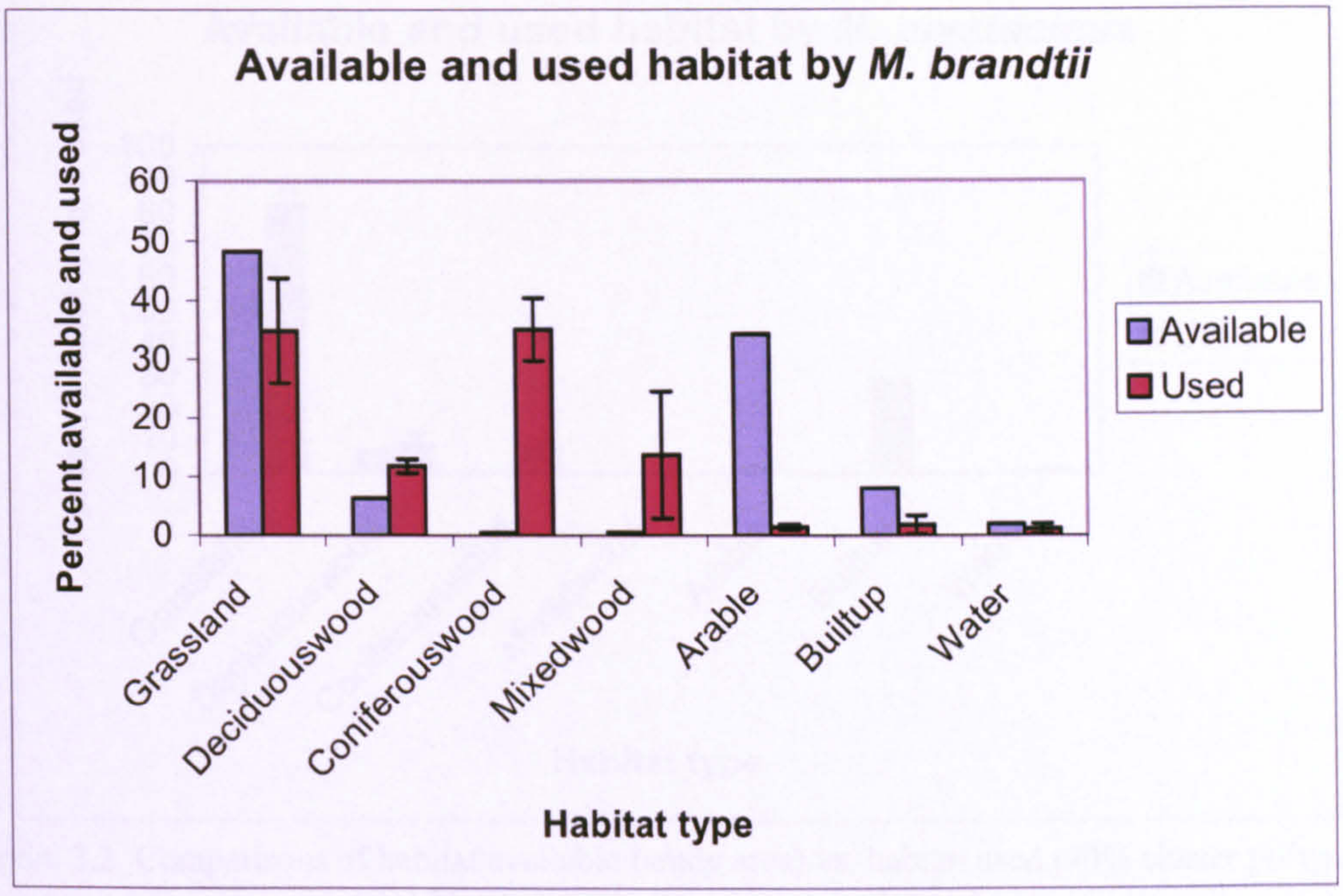

Figure 3.1. Comparisons of habitat available (study area) vs. habitat used (90% cluster polygons) by *M. brandtii* (n=11) (bars = standard error)

	Grassland	Deciduous woodland	Coniferous woodland	Mixed woodland	Arable land	Builtup areas	Water	Rank
Grassland		+	-	-	+++	+++	+	4
Deciduous woodland	-		-	-	+++	+++	+	3
Coniferous woodland	+	+		+	+++	+++	+	6
Mixed woodland	+	+	-		+++	+++	+	5
Arable	---	---	---	---		-	---	0
Builtup	---	---	---	---	+		-	1
Water	-	-	-	-	+++	+		2

Table 3.5. Simplified ranking matrix for 11 *M. brandtii* based on comparing proportions of habitat within the study area (available habitat) and 90% cluster polygons (used habitat)

The signs show whether the habitat category placed in the corresponding row was more (+) or less (-) important than the corresponding column of the matrix. A triple sign (+++ or ---) indicates a significant ($P < 0.05$) difference between the two habitat categories. One sign (+ or -) shows a non-significant trend. Relative importance of the different habitat categories (Rank) was determined by the number of + and +++ signs occurring in the rows. Habitats were ranked according to their importance from zero (least important) to 7 (most important) Significance levels from t-tests on the observed data is shown in parenthesis where it differs.

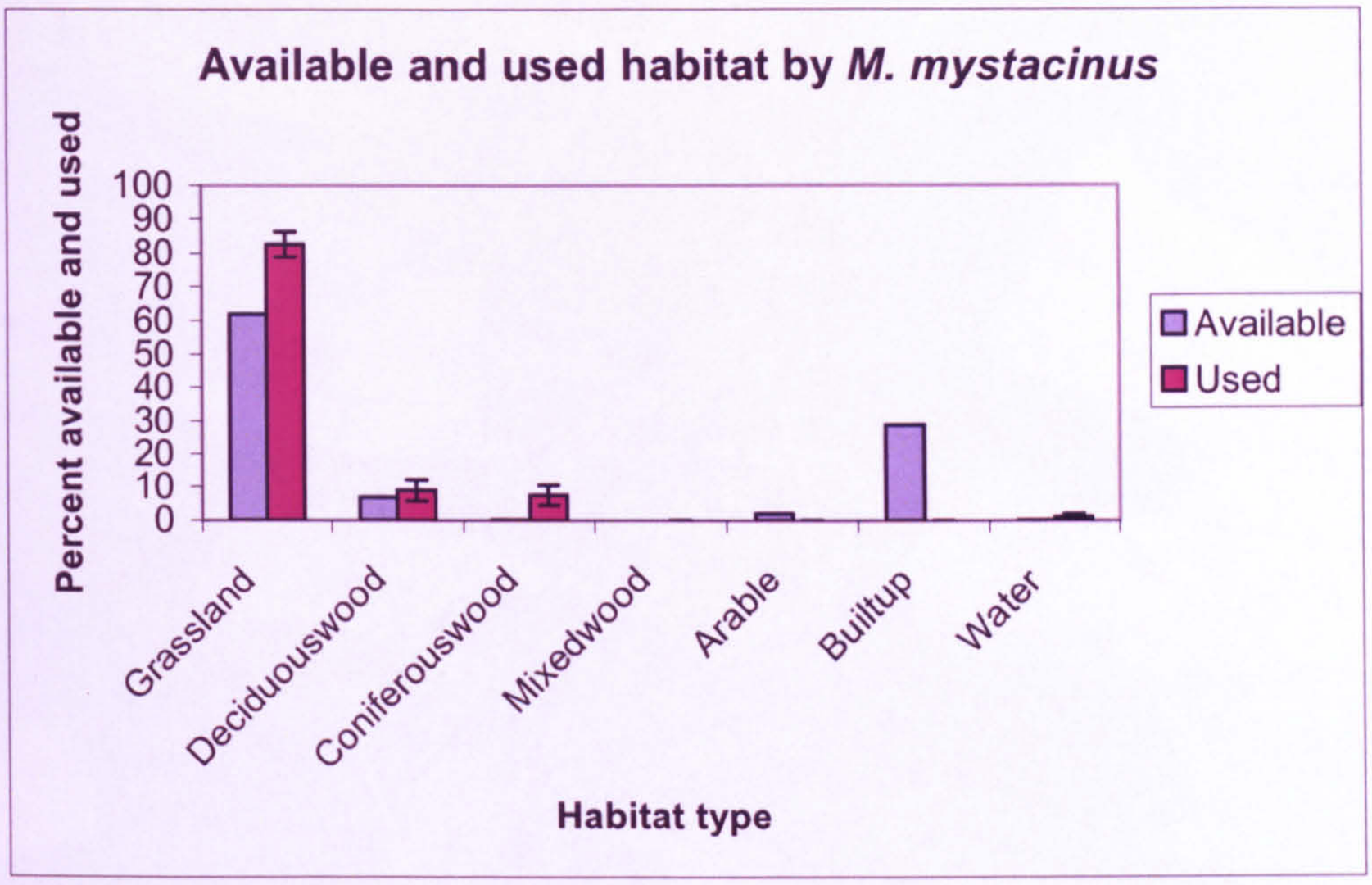

Figure 3.2. Comparisons of habitat available (study area) vs. habitat used (90% cluster polygons) by *M. mystacinus* (bars = standard error)

	Grassland	Deciduous woodland	Coniferous woodland	Arable land	Builtup areas	Rank
Grassland		+	+++	+++	+++	4
Deciduous woodland	-		+	+	-	2
Coniferous woodland	---	-		+	---	1
Arable land	---	-	-		---	0
Builtup areas	---	+	+++	+++		3

Table 3.6. Simplified ranking matrix for 9 *M. mystacinus* based on comparing proportions of habitat within the study area (available habitat) and 90% cluster polygons (used habitat)

The signs show whether the habitat category placed in the corresponding row was more (+) or less (-) important than the corresponding column of the matrix. A triple sign (+++ or ---) indicates a significant ($P < 0.05$) difference between the two habitat categories. One sign (+ or -) shows a non-significant trend. Relative importance of the different habitat categories (Rank) was determined by the number of + and +++ signs occurring in the rows. Habitats were ranked according to their importance from zero (least important) to 4 (most important).

Plate 3.1. MCP (yellow) and 90% cluster polygon (blue) of 1 *M. brandtii*
(the red dot indicates the main study colony)

Plate 3.2. MCP (yellow) and 90% cluster polygon (blue) of 1. *M. mystacinus*
(the red dot indicates the main study colony)

Plate 3.3. Key foraging habitat of *M. brandtii*

Plate 3.4. Key foraging habitat of *M. mystacinus*

4. NOCTURNAL ACTIVITY

ABSTRACT

12 *M. mystacinus* and 12 *M. brandtii* were radiotracked in 2003-2004 in order to investigate whether these cryptic species have different nocturnal activity. Their similar morphologies suggest that their foraging ecologies will also be similar.

Night roost fidelity, time of last return, number of foraging bouts per night, maximum foraging distance, minimum convex polygon (MCP) size and size of core foraging areas were similar for the two species. However, *M. mystacinus* emerged earlier than *M. brandtii* and changed day roosts less frequently. *M. mystacinus* also showed greater foraging site fidelity, less foraging site overlap and had longer flying times than *M. brandtii.* Differences in habitat quality, roost characteristics or the bats' individual hunting abilities may have influenced the results. However, two study colonies were used for each species with similar results, so inter-specific differences in nocturnal activity are more likely to be general ecological differences. The longer flying times for *M. mystacinus* in spite of having less foraging sites than *M. brandtii* may suggest that coniferous woodland, the preferred foraging habitat of *M. brandtii,* is of better quality for foraging bats in the UK than previously assumed. These differences in nocturnal activity therefore show that the cryptic species, although morphologically similar, occupy different ecological niches and consequently need different management in order to be conserved effectively.

4.1. INTRODUCTION

At present there is little information concerning the conservation status of the cryptic species *M. mystacinus* and *M. brandtii*. Although the "Action Plan for the Conservation of Bats in the United Kingdom" highlights that "further research is needed to establish ecological and conservation requirements of either species" (Hutson 1993), little research has been directed towards the ecology of the two species. Consequently there is an urgent need for the foraging behaviour of each species to be described, especially since many cryptic species can show considerable ecological differences despite morphological similarity (e.g. Arlettaz 1996, Barlow 1997).

Understanding factors influencing the home-range requirements within populations is important for defining areas required to maintain viable populations. Information relating to foraging distances of greater horseshoe bats (*Rhinolophus ferrumequinum*) in south west England (Duvergé and Jones 1994) has been used successfully to target agri-environment schemes in order to improve habitat around known maternity colonies of this species (Longley 2003).

Radiotracking has been used in a large number of studies of bats in order to study aspects of their ecology such as habitat use, movements, home-range requirements and foraging behaviour (e.g. Jones and Morton 1992, O'Donnell 2001, Bontadina et al. 2002). However, the additional weight of radio transmitters on flying animals has consequences for both their energetic costs and their manoeuvrability (Caccamise and Hedin 1985, Hughes and Rayner 1991), consequently, there has been some debate on whether the transmitters influence the bats' behaviour significantly (Rayner et al. 1989, Hickey 1992). A maximum mass of radiotransmitters of 5% of the body mass was recommended by Aldridge and Brigham (1988) for flying animals. Hence, the small body mass of *M. mystacinus* and *M. brandtii* (3.5 -9g.) has previously excluded the use of radiotracking as the lightest transmitters exceeded the recommended surplus weight they added to the animals. However, recent technological advances have reduced the mass of radio transmitters to an acceptable level for tagging the species. Refer to section 3.2. for further discussion on the influence of radiotagging on bat behaviour.

The major aim of this study was to investigate the nocturnal activity of *M. mystacinus* and *M. brandtii*. Because most nocturnal activity probably involves flights to foraging areas, my findings will assist in the development of conservation strategies that can take into account the appropriate management of feeding sites for these cryptic species.

4.2. METHODS

Refer to section 3.2. for information on study sites, choice of method, capturing, tagging and radiotracking of bats.

Digital maps were downloaded from Digimap (www.edina.ac.uk/digimap/) and imported into the GIS software Arc View 3.1 (ESRI UK) using the software MapManager 6.2 (ESRI, UK). Data analysis mostly followed the methods of Jones and Morton (1992), O'Donnell (2001) and Bontadina et al. (2002). Radiotracking data for each species were displayed and analysed using ArcView. Home ranges have been expressed as 100% minimum convex polygons (MCPs) to facilitate comparison with other studies (Harris et al.1990). To avoid pseudo replication one average point per bat was used. Mann- Whitney U statistical tests were carried out to test for significant differences between the species. This test was chosen over ANOVA after an Anderson- Darling test showed that some of the data were not normally distributed, in addition to the sample size being small. In all statistical tests significance was set at $P < 0.05$ and the software MiniTab 14 was used for statistical analysis.

4.3. RESULTS

9 *M. mystacinus* were captured and radiotracked around the colony at Compton House (Bristol) and 3 bats were captured and radiotracked around the colony at Stoford Manor (Taunton). 11 of the *M. brandtii* were captured and radiotracked around the colony at Golden Mill (Truro), the remaining 1 bat was captured and radiotracked around the colony at Church House (South Brent). Hence, a total of 12 *M. mystacinus* and 13 *M. brandtii* were fitted with radiotransmitters in 2003 and 2004 (1 *M. brandtii* was not radiotracked due to tag failure and/or the bat moving away from the study area).

The radiotransmitters attached to the 12 *M. mystacinus* from both study colonies had a life of 3- 14 days, with an average of 7.83 days, but this is difficult to estimate as some transmitters were still working when the radiotracking session ended. The radiotransmitters attached to the 12 *M. brandtii* from both study colonies had a life of 3-11 days, with an average of 7.58 days. The 12 *M. mystacinus* were radiotracked for a total of 78 bat nights, with an average of 6.5 nights per bat and a total of 47 bat nights with good data, and an average of 3.92 nights with good data (defined as being able to stay in contact with the bat 90% of the time from emergence to last return) per bat. The 12 *M. brandtii* were radiotracked for a total of 80 bat nights, with an average of 6.67 nights per bat and a total of 58 bat nights with good data, and an average of 4.83 nights with good data per bat. For further information about duration of radiotracking of the bats from both main and secondary study colonies, refer to Table 4.1.

All the radiotracked bats were adult females, except one adult male *M. mystacinus*. Five of the *M. brandtii* and 6 of the *M. mystacinus* showed evidence of current lactation when they were radiotagged. The bats were radiotracked at similar times of the year (U=120.0, N_1=N_2=12, P= 0.0867). For general radiotracking information e.g. sex, colony information and tracking dates, refer to Table 3.3.

4.3.1. Roosting

Roost and night roost fidelity is summarised in Table 4.2. Number of day roosts was significantly higher for *M. brandtii* (mean 1.83) than *M. mystacinus* (1.33) (U=188.0 N_1=N_2=12, P= 0.0133). Out of the total number of *M. brandtii* 68.18% of the bats selected houses or cottages, 22.7% of the bats selected barns or outbuildings, 4.55% of the bats selected farmhouses and 4.55% selected tree roosts of unknown species as day roosts. For *M. mystacinus*, on the other hand, 71.43% of the bats selected houses or cottages as their day roosts, 14.29% of the bats selected barns or outbuildings and 14.29% of the bats selected farmhouses as their day roosts. The two species therefore seem to use houses or cottages to a similar degree, but *M. brandtii* select barns, outbuildings and trees as day roosts to a greater degree than *M. mystacinus* (Figure 4.1).

The mean number of night roosts was similar for *M. brandtii* (1.75) and *M. mystacinus* (1.42) (U=172.0, N_1=N_2=12, P= 0.1661). Out of the total number of *M. brandtii* 47.62% of the bats selected barns or outbuildings, 38.10% of the bats selected houses or cottages, 4.76% of the bats selected farmhouses and 9.52% selected tree roosts of unknown species as night roosts.

For *M. mystacinus*, on the other hand, 64.71% of the bats selected houses or cottages as their night roosts, 23.53% of the bats selected farmhouses and 11.76% of the bats selected barns or outbuildings as their night roosts. *M. brandtii* therefore seem to night roost more in barns, outbuildings and trees and less in houses, cottages and farmhouses than *M. mystacinus* (Figure 4.2). Due to access problems and lack of personnel, it was not possible to determine where the bats roosted in all the buildings or in which tree species the tree roost was located. However, for the roosts where access was possible or the exit points of the roost could be determined the bats seemed to be roosting in the attic space or under the roof tiles.

4.3.2. Flying behaviour

Flying behaviour is summarised in Table 4.2. Overall *M. mystacinus* emerged 10.05 minutes before *M. brandtii*, on average 33.20 and 43.25 minutes after sunset respectively. The difference was statistically significant (U=199.0. N_1=N_2=12, P= 0.0051). *M. mystacinus* had a significantly longer average flying time of 276.9 minutes, ranging from 142 to 320 minutes, than *M. brandtii*, which had an average flying time of 248.1 minutes, ranging from 236 to 318 minutes (U=111.0, N_1=N_2=12, P= 0.0262).

Number of foraging bouts per night was similar in *M. brandtii* and *M. mystacinus* (U=146.5, N_1=N_2=12, P= 0.8625), averaging 1.57 and 1.55 bouts respectively.

Overall *M. mystacinus* returned 8.58 minutes before *M. brandtii*, on average 126.93 and 135.51 minutes before sunset respectively, and return time was not significantly different between the species (U=154.0, N_1=N_2=12, P= 0.8399).

4.3.3. Foraging sites

Results for foraging sites is summarised in Table 4.2. While on average *M. brandtii* had 2.5 foraging sites per bat, *M. mystacinus* used fewer foraging sites, 1.25, per bat. This was significantly different between the species (U=199.5, N_1=N_2=12, P= 0.0022).

Core foraging areas (90% cluster polygons) ranges from 1.37- 10.66 ha for *M. mystacinus* with an average of 5.44 ha and ranges from 1.01- 10.89 ha for *M. brandtii,* with an average of 4.26 ha. There was no significant difference between the size of core foraging areas (U=130.5, N_1=N_2=12, P= 0.2724). Refer to Plate 4.1 and 4.2 to compare differences in core foraging areas (90% cluster polygons) of *M. mystacinus* and *M. brandtii.*

Maximum foraging distance was similar for *M. mystacinus* and *M. brandtii* (U=134.0, $N_1=N_2=12$, P= 0.3699), ranging from 200 to 2300 m for *M. mystacinus*, with an average of 812 m; and from 300 to 3200 m, with an average of 791 m for *M. brandtii.*

MCP size ranges from 13.2- 119.5 ha for *M. mystacinus,* with an average of 40.7 ha and ranges from 5.4- 257.8 ha, with an average of 42.9 for *M. brandtii.* The difference was not statistically significant (U=166.0, $N_1=N_2=12$, P= 0.3708).

4.4. DISCUSSION

4.4.1. Roosting

Both *M. mystacinus* and *M. brandtii* changed day and night roosts, with *M. brandtii* changing day roosts significantly more often even though the two species were radiotracked for similar lengths of time. Neither species showed any strong evidence of changing roosts in order to minimise commuting distance to foraging areas. Roost switching behaviour has also been found in studies of other species e.g. *R. ferrumequinum (*Jones and Morton 1992) and *Nyctalus leisleri* (Waters et al. 1999). Lewis (1995) found that high roost fidelity was directly related to roost permanency and inversely related to roost availability. Lewis (1996) found that *Antrozous pallidus* changed roosts more often if parasite loads were high, however, parasite loads appeared to be low for *M. mystacinus* and *M. brandtii* and their body mass indicated that they were in good condition in comparison to previous studies on the two species (Baagøe 1973, Jones 1991, Chapter 2). Temperature has also been found to influence roost fidelity. *Eptesicus serotinus* are strongly philopatric to their roosts (Catto et al. 1996, Harbusch and Racey 2006) and Harbusch and Racey (2006) suggested that this may partly be due to the range of different microclimates. *Myotis nattereri* seemed to require a large number of roosts with different temperatures (Smith and Racey 2005b). *Pipistrellus pygmaeus* was found to leave bat boxes on the hottest days (Lourenco 2004). This is likely to also be the case with *M. mystacinus* and *M. brandtii* as colonies which were not radiotracked often disappeared to unknown locations. Russo et al. (2004) found that *Barbastella barbastellus* females changed roosts less frequently during lactation. Effects of reproductive stage could not be analysed in my study due to the small sample size.

The radiotracked bats were only found roosting in buildings (different types of private dwellings, barns and outbuildings), except from 1 *M. brandtii* which used a tree as day roost for 2 days and 2 *M. brandtii* which spent shorter periods night roosting in trees. *Plecotus auritus* (Entwistle et al. 1997) and *M. nattereri* and *P. pipistrellus* show roost selectivity (Smith and Racey 2005b) in structural or habitat attributes of roosts. It is likely that this is also true for *M. mystacinus* and *M. brandtii* although this was not investigated in my study. It is likely that they use tree roosts to a greater extent than is currently known because only 9 main maternity colonies of each species could be located in England from bat group contacts, even though bats of these species are regularly caught in harp traps and mist nets (L. Berge unpublished). Nyholm (1965) in a study of *M. mystacinus* in Finland found that the bats used 75% manmade structures for roosting further supporting this hypothesis.

4.4.2. Flying behaviour

4.4.2.1. Emergence

My radiotracking showed that overall *M. mystacinus* emerged 10 minutes before *M. brandtii*, on average 33.2 and 43.3 minutes after sunset respectively. The difference was statistically significant. An Irish study found that the emergence time of *M. mystacinus* ranged from 23 to 49 minutes after sunset, the mean emergence time was 34.7 minutes (Buckley 2004). Buckley (2004) found no significant correlation between the first emergence and temperature or night length. Another emergence study found that *M. mystacinus* emerge within 30 minutes of sunset (Jones and Rydell 1994). Hollyfield found that the first emergence of *M. mystacinus* was on average 24.11 minutes after sunset and was highly correlated with sunset time. Wind speed, light levels and minimum and maximum temperatures were not correlated with emergence (Hollyfield 1993). Note that a more northerly distribution may cause differences in emergence times (Catto et al. 1995), this is not likely to influence the results from the studies mentioned above to a great extent however. There are no published studies on the emergence behaviour of *M. brandtii.* However, emergence times on a range of other bat species can be found in Jones and Rydell (1994). When comparing the emergence times of UK bat species one can see a pattern in that larger species emerge early and smaller species and gleaners emerge late. This is probably correlated to flight behaviour, foraging habitat and diet on one hand and predator avoidance and maximisation of feeding on the other hand. This is because early emergence increases the risk of predation by raptorial birds and late emergence may lead to the bats missing the dusk peak of aerial insects. Unsurprisingly however, Petrzelkova

(2003) found no difference in the emergence of *Eptesicus nilsonii* with the presence of a trained barn owl (*Tyto alba*), only a larger degree of clustering. On the other hand, studies have shown that *P. auritus* emerge very late, on average 61.7 minutes after sunset (Entwistle et al. 1997); this is thought to be due to their main prey (moths) not having a peak in availability around dusk (Rydell et al. 1996) unlike many other aerial insects. Swift (1980) found a strong linear relationship between colony size and average rates of emergence in *Pipistrellus* spp., suggesting that the larger the number of bats using a roost, the longer the average time of emergence. The ability to leave the roost with large numbers of other bats present may therefore be a significant constraint to species in such colonies and therefore the cost of belonging to a large colony may reduce foraging time (Avery 1986). Colonies of *M. mystacinus* and *M. brandtii* however are generally quite small, the four colonies used in my study had 25- 80+ adult bats, it is therefore unlikely that colony size will have a great effect on emergence timing in these colonies.

Although my study shows a significant difference between the emergence times of *M. mystacinus and M. brandtii*, it is likely that this behaviour is also influenced by other factors that were not incorporated as part of this study, such as roost attributes, although the use of two study colonies for each species reduces this possibility. For example, emergence times of *P. pygmaeus* and *E. serotinus* (Catto et al. 1996, Downs et al. 2003) were affected by external illumination near their roost exit and tree cover near roosts influenced emergence times of *Pipistrellus* spp. (Jenkins et al. 1998). In particular, bats emerge earlier from exits that are close to shelter (Entwistle et al. 1997, Duvergé 2000). Note that Jones (1995) points out that lactating females tend to emerge earlier; but this could not be investigated in my study due to the small sample size.

4.4.2.2. Flying time

M. mystacinus has significantly higher average flying times than *M. brandtii* (276.9 and 248.1 minutes respectively). The range was large, from 142 to 320 minutes. Davidson-Watts and Jones (2006) and Nicholls and Racey (2006a) found that flying time was significantly different for the cryptic species *P. pipistrellus* and *P. pygmaeus*. Nicholls and Racey (2006a) conclude that these differences are probably due to habitat fragmentation of the open agricultural landscape utilised by the *P. pipistrellus*, with a higher patch dispersion necessitating larger range sizes and hence a higher flying time to catch the necessary amount of prey. It may also be due to differences in insect availability at their foraging sites. Quality

of surrounding habitat probably plays a major role in the flying time of the bats, in addition to the hunting abilities of individual bats. It is also important to keep in mind that late in the season the bats may be mating and not just flying (Davidson-Watts and Jones 2006). Encarnacao et al. (2006) found that the duration of nocturnal activity of *M. daubentonii* varied over the season and was most extended in mid summer, while *R. ferrumequinum* fed for (2.25) hours per night early in the season, increasing to 4 hours after given birth in July (Duvergé and Jones 1994). Similar seasonal differences are likely with *M. mystacinus* and *M. brandtii* although this could not be investigated in my study due to the small sample size. One would expect *M. brandtii* having longer average flying times due the bats having more foraging areas (probably indicating that they exploit more patchily distributed prey in their habitats). *M. brandtii* also forage in habitats (coniferous woodland) that are generally thought of being of lower quality than grassland (especially pasture), which was found to be the primary foraging habitat of *M. mystacinus* (Chapter 3). Surprisingly, *M. mystacinus* has longer flying time than *M. brandtii*. This may be due to random differences in hunting abilities between individual bats or coniferous woodland being of better quality as foraging habitat for bats then previously expected.

4.4.2.3. Return

Overall *M. mystacinus* returned 8.6 minutes before *M. brandtii*, on average 126.9 and 135.5 minutes before sunset respectively, there was no significant difference between the two species. There are no published studies on the return timing of the two species, but Davidson-Watts and Jones (2006) found differences in return time between the cryptic species *P. pipistrellus* and *P. pygmaeus* (177.8 and 268.8 minutes before sunrise respectively) and related this to differences in habitat quality or hunting ability of individual bats.

4.4.2.4. Flying behaviour

Differences have been found between flying time of *Pipistrellus* spp. in the UK (Swift 1980, Maier 1992, Davidson-Watts and Jones 2006, Nicholls and Racey 2006a). Such differences may be due to climatic variation (Kunz 1982). Hence, it is also likely that there will be differences between studies in different geographic areas for *M. mystacinus* and *M. brandtii* due to their widespread distribution.

Weather conditions (wind, rainfall, humidity, moonlight), metabolic demands, predation pressure and inter-specific competition all influence the emergence timing of bats, and the

duration and patterns of their activity (see reviews in e.g. Rydell et al. 1996, Vaughan et al. 1997). Most of these influences were not investigated in my study. However, Stebbings (1968) reported that rain did not interfere with the nocturnal activity of *P. pipistrellus* in England and cited Williams (1940), who found that precipitation did not decrease the numbers of insects coming into a light trap. Similar results were found in a study of *M. lucifugus* where the females emerged even during an electrical storm (Fenton 1990). Bad weather conditions such as heavy rain did not seem to influence the foraging behaviour of the bats significantly in my study because even during very heavy rain the bats would still be foraging rather than returning to the roost. Entwistle et al. (1997) came to a different conclusion in a study of *P. auritus* where they found that rain inhibited emergence and flight activity.

4.4.2.5. Foraging bouts

Nyholm (1965) noted in a study of *M. mystacinus* that there were intervals during the night when the bats hung off a tree trunk, dry branch stump, vertical rock wall or live branch. The lengths of these breaks were irregular. A German radiotracking study of 9 *M. brandtii* females however, showed that the bats had one single period of activity from dusk until dawn (Dense and Rahmel 2002). I found that the bats often had several foraging bouts per night with the number of foraging bouts being similar for *M. brandtii* and *M. mystacinus*, 1.77 and 1.75 respectively. These foraging bouts were often immediately after dark and before sunset. Patterns of bat activity showing foraging peaks immediately after dark and before dawn have been widely reported (e.g. Herreid and Davis 1966, Fenton 1970, Kunz 1973, 1974, Funakoshi and Uchida 1975). *R. ferrumequinum* had a first feeding sessionon lasting on average 1.25 hours. Then they night roosted for 1-3 hours, and then had another feeding session (Duvergé and Jones 1994). Davidson-Watts and Jones (2006) found that *P. pipistrellus* had 1.46 foraging bouts while *P. pygmaeus* had 1.14 foraging bouts. *Nyctalus leisleri* had 1.38 foraging bouts on the other hand (Waters et al. 1999), while *P. auritus* was found to forage actively throughout the night (Entwistle et al. 1997). These differences between species may be due to differences in diet and foraging behaviour. There is a general consensus that the two activity peaks coincide with peaks in aerial insect activity (Swift 1980, Racey and Swift 1985, Swift and Racey 1983, Taylor and O'Neill 1988, Richards 1989, Barclay 1991, Rydell et al. 1996). However, no bimodal pattern was found in *Chalinolobus tuberculatus*, but this could be an adaptation to a high rainfall area (O'Donnell 2001). Differences in numbers of foraging bouts have also been found between different stages of

reproduction. Swift (1997) found that *M. nattereri* had 1.0 foraging bouts during pregnancy, 1.84 during early lactation and 1.0 foraging bouts post lactation. Nicholls and Racey (2006a) found similar results for *P. pipistrellus* and *P. pygmaeus* at different stages of reproduction. The bats radiotracked outside the lactation period typically had a single foraging bout, while during lactation they had two or three foraging bouts per nights. Similar patterns were also observed in *P. pipistrellus* by Swift and Racey (1983) during lactation and weaning, and insect activity patterns in the area showed similar peaks. However, during pregnancy and after weaning, the patterns differed in that most bats foraged only once per night and then returned to the roost. There was no significant difference between the number of foraging bouts for *M. mystacinus* and *M. brandtii*. Due to the small sample size, it was not possible to look into difference between the different reproduction states of *M. mystacinus* and *M. brandtii*.

Night roosting between foraging flights is a common habit of temperate zone insectivorous bats (e.g. Barclay 1982). Several authors report that night roosting is more common after the active maternity period e.g. Davis et al. (1968) for *Eptesicus fuscus* and O'Shea and Vaughan (1977) for *A. pallidus*. Also, insect resources remain spatially and temporally variable, and bats spend less time foraging and more time roosting when insect density is low or cool ambient temperatures prevail. This relationship implies that bats cease foraging when poor foraging success and/or high costs of flight and thermoregulation prevent the maintenance of a positive energy balance.

4.4.3. Foraging sites

4.4.3.1. Foraging site fidelity

Little is known about whether bats partition their foraging ranges, or how they space themselves to minimise potential competition. Although there is evidence for inter-specific partitioning of space in bats (e.g. Aldridge and Rautenbach 1987, Crome and Richards 1988, Arita and Fenton 1997), conclusions from studies investigating intra-specific behaviour have been vague (Fenton and Bell 1979, Bell 1980). Patterns of home range spacing within bat species include territoriality (e.g. Bradbury and Emmons 1974) and use of exclusive feeding areas through mutual avoidance (e.g. Vaughan 1976, Leonard and Fenton 1982). Colonial and individual home ranges overlapped in *Eptesicus serotinus* (Robinson and Stebbings 1997). Swift and Racey (1983) on the other hand, noted that maintenance of exclusive foraging areas by *Pipistrellus* spp. only became evident when insect densities at a site were low. Rydell

(1986) described the same behavioural changes in *E. nilsonii* in Sweden, as did Belwood and Fullard (1984) for *Lasiurus cinereus semotus* in Hawaii. Most *Rhinolophus ferrumequinum* also had preferred feeding sites and flew straight to these (Duvergé and Jones 1994). Use of individual foraging areas is also known in e.g. *Euderma maculatum* (Wai-Ping and Fenton 1989), *Eptesicus fuscus* (Brigham 1988) and *Myotis bechsteinii* (Kerth et al. 2001). On the other hand, several species have been found to share foraging sites and have sometimes even been found to forage in cohesive flocks e.g. *M. lucifugus* (Barclay 1982), *P. pipistrellus* (Racey and Swift 1985), *Nyctalus humeralis* (Wilkinson 1992), *E. nilssoni* (De Jong 1994), *E. serotinus* (Catto et al. 1996), *M. myotis* and *M. blythii* (Arlettaz 1996, 1999), *Nyctalus leisleri* (Shiel et al. 1999). The reason for this flocking behaviour is unknown for most of the species. However, flock-feeding appears to be correlated with an aerial-hawking strategy and clumped distribution of resources (Nicholls and Racey 2006a).

I found that *M. mystacinus* usually had one foraging area and only one radiotracked bat used each foraging area, however, the radiotracked *M. brandtii* had several foraging sites and switched between these sites, with several of the radiotracked bats using the same foraging site. The difference in number of foraging areas between species was statistically significant. The gleaning bats *M. bechsteinii* and *P. auritus* both use specific foraging sites (Entwistle et al. 1997, Kerth et al. 2001). Similar behaviour can be found in other Palaearctic bat species (e.g. Racey and Swift 1985, Rydell 1989, Audet 1990, Brigham 1991). Catto et al. (1996) found that individual *E. serotinus* used up to five foraging sites per night, while Nicholls and Racey (2006a) found no significant difference between the number of core areas for *P. pipistrellus* and *P. pygmaeus* (Entwistle et al. 1997). Only two of the studies looked into whether individual bats are loyal to their foraging sites in different seasons and years. A study of *M. myotis* found that bats radiotracked in different seasons continued to use the same foraging sites (Audet 1990) while a radiotracking study of *M. bechsteinii* found that the bats used the same foraging sites between different years (Kerth et al. 2001). Differences between years by the same bat were not investigated in my study. Differences between numbers of foraging sites and foraging site fidelity may be due to differences in food predictability and availability, with species having less foraging sites and showing greater foraging site fidelity foraging on predicable resources (Nicholls and Racey 2006a). Differences between individual and group foraging on the other hand, may be due to long duration of food availability and an even distribution of food patches leading to individual foraging, while clumped, ephemeral food can select for group foraging and information transfer (Nicholls and Racey 2006a).

Uniform food distribution and predictable prey availability may cause foraging site fidelity over several seasons and years (Irons 1998). Strong fidelity to distinct individual feeding sites over several seasons suggests that the distribution of food is uniform and prey availability is predictable (Irons 1998).

4.4.3.2. Home range size

The main hunting grounds of *M. brandtii* were found to be within 1.5 km to over 10 km away from the maternity colony (Dense and Rahmel 2002). The maximum foraging distance in my study however, was 3.2 km for *M. brandtii* and 2.3 km for *M. mystacinus*. The mean foraging distance was around 0.8 km for both species. There were great differences in the maximum foraging distance for each individual bat in my study, with some bats having a maximum foraging distance as low as 0.2 km. The maximum foraging distance of *M. mystacinus* and *M. brandtii* is much smaller than for most other European bat species e.g. *M. bechsteinii*, range 0.05- 1.0 km (Kerth et al. 2001); *Plecotus auritus* maximum 1.1 km (Swift and Racey 1983); *P. pipistrellus* mean 1.7 km (Davidson-Watts and Jones 2006); *P. pygmaeus,* mean 1.5 km (Davidson-Watts and Jones 2006); *R. ferrumequinum*, mean 2.85 km (Duvèrge and Jones 1994); *M. daubentonii*, maximum 2.3 km, mean 1.4 km, range 0.6- 6.3 km (Dietz et al. 2006); *Rhinolophus hipposideros*, maximum 4.17 km, minimum 0.24 km with 50% of the locations being within 0.6 km of the roost (Bontadina et al. 2002); *N. leisleri,* maximum 5.75 km, minimum 2.88 km, mean 4.2 km (Waters et al. 1999); *E. serotinus*, mean 7.4 km (Robinson and Stebbings 1977) and 6.5 km (Catto et al. 1996) and *Barbastella barbastellus* range 2-18 km (Greenaway 2001).

MCP size is similar for the two species and ranges from 13.2- 199.5 ha for *M. mystacinus,* with an average of 40.7 ha and ranges from 5.4- 257.8 ha, with an average of 42.9 ha for *M. brandtii.* Core foraging areas (90% cluster polygons) ranges from 1.37- 10.66 ha for *M. mystacinus* with an average of 5.44 ha and ranges from 1.01- 10.89 ha for *M. brandtii,* with an average of 4.26 ha. These are the first studies on the MCP sizes of the two species. In a similar study on the cryptic species *Myotis myotis* and *M. blythii,* there was no difference in the size of their foraging areas (Arlettaz 1996). However, in a study of *P. pipistrellus* and *P. pygmaeus* in southern England Davidson Watts and Jones (2006) found an average MCP size of 157 and 146 ha respectively, 90% cluster polygons were on average 11.5 and 4.6 ha respectively. In a study of *R. hipposideros* MCP sizes ranged from 1- 368 ha, while 100% kernel sizes ranged from 12-53 ha (Bontadina et al. 2002). Large bats tend to have larger

foraging areas. In a study of *E. serotinus* there were large differences in MCP size ranging from 0.16 km^2 to 47.6 km^2, with a mean size of 7.46 km^2 (Robinson and Stebbings 1997). *N. leisleri* also showed differences in MCP sizes ranging from 2.42 to 18.36 km^2 with a mean of 7.4 km^2 (Waters et al. 1999). The mean MCP sizes of *M. bechsteinii* however, ranged from 9.9- 37.5 ha (Kerth et al. 2001).

Home-range size is predicted to be dependent on the spatial dispersion of resource patches. This is because in order to ensure sufficient amounts of foods, a certain number of resource patches must be included in an animal's home range (Carr and Macdonald 1986). In habitats where prey availability varies greatly, additional foraging areas need to be included in the animal's range. Resource distribution therefore has a greater influence on home range size than species richness (Macdonald 1983). It is therefore possible that sufficient and predictable resources explain some of the great differences in home range sizes and foraging distances compared to most other European bat species. Note that the two other European bat species mentioned earlier with small ranges (*P. auritus* and *M. bechsteinii)* are gleaners and gleaned prey may represent a more predictable resource than aerial insects (Arlettaz 1999). Jones et al. 1995 concluded that a high foraging distance is related to a high aspect ratio because this relates to low commuting costs; additionally high wing loading also gives fast flight and hence reduces time spent on migration making commuting less costly (Norberg and Rayner 1987). This corresponds well with the results for European bat species with bats having a low wing loading and low aspect ratio having short maximum foraging distances and small ranges, while bats with a high wing loading and high aspect ratio have high maximum foraging distances and large home ranges. Both *M. mystacinus* and *M. brandtii*, in addition to the two other European bat species with small ranges discussed earlier (*P. auritus* and *M. bechsteinii)* all have relatively low wing loadings and aspect ratios (Norberg and Rayner 1987). Note that the range estimations for *M. mystacinus* and *M. brandtii* should be viewed with slight caution however, as bats were lost occasionally and could not be found until they returned to their previous feeding area. It is therefore likely that their foraging distance, MCP size and core area size would increase if the bats were not lost for parts of the night. It is important to keep in mind that only radiotracking nights with good data (defined as being able to stay in contact with the bats for 90% of the time from emergence to sunrise) were used for analysis in the study. The extent of which the ranges would increase if contact with the bat could be maintained throughout the radiotracking period would only be speculation. Still,

their foraging ranges would still be expected to be relatively small compared to most other European bat species.

4.5. CONCLUSION

This study has shown differences in nocturnal activity between the two species. Most interestingly, *M. mystacinus* seem to be more territorial and loyal to their foraging areas. *M. mystacinus* also emerge earlier, change day roosts less frequently than *M. brandtii* and have longer flying times. Night roost fidelity, time of last return, number of foraging bouts per night, maximum foraging distance, MCP size and the size of core foraging areas were similar for the two species. There are few studies on the foraging behaviour of the two species and differences could be due to differences in habitat quality, prey availability and predictability, roost characteristics or individual bats' hunting abilities rather than general ecological differences. However, bats from two study colonies in different geographic areas were radiotracked for each species with very similar results although this could not be tested statistically due to the small sample sizes from the secondary colonies. For a comparison of results between the main and secondary study colonies for each species, refer to Table 4.4 and 4.5. An additional radiotracking study should be carried out where both *M. mystacinus* and *M. brandtii* are radiotracked in the same geographical area simultaneously. A study using a larger sample size and tracking males and females in different reproductive stages of both species at a range of different sites in different geographical areas could also provide some interesting and useful results.

My results show that although *M. mystacinus* and *M. brandtii* have almost identical morphologies, their nocturnal activity is different in some respects such as emergence timing, flying time, roost fidelity, foraging site fidelity and foraging site overlap. These differences are likely to arise from differences in habitat use with *M. mystacinus* being most reliant on grassland (particularly pasture surrounded by hedgerows, often grazed pasture) while *M. brandtii* is more associated with woodland, typically coniferous woodland (see Chapter 3) and differences in prey availability and distribution within these habitat types. This may also suggest that coniferous woodland is a habitat better suited for bat foraging than previously assumed. This study has therefore shown that bat species may therefore, although similar in

morphology, have similar nocturnal activity and consequently need different management plans.

Variable	Species	Range	Mean
Number of days tracked	*M. brandtii*	3.0- 10.0	6.67
	M. mystacinus	3.0- 10.0	6.50
Number of days with good data	*M. brandtii*	2.0- 10.0	4.83
	M. mystacinus	3.0- 7.0	3.92
Tag life, number of days	*M. brandtii*	3.0- 11.0+	7.58+
	M. mystacinus	3.0- 14.0+	7.83+

Table 4.1. Range and mean of number of days tracked, number of days with good data and tag life for *M. mystacinus* and *M. brandtii* from two study colonies for each species (n= 24, 12 and 12 respectively), with good data defined as being able to stay in contact with the bat for 90% of the time from emergence until last return

Variable	Species	Range	Mean	Median	S.D.
Number of day roosts	*M. brandtii*	1.0- 2.0	1.83	2	0.701
	M. mystacinus	1.0- 3.0	1.33	1	0.405
Number of night roosts	*M. brandtii*	1.0- 3.0	1.75	2	0.647
	M. mystacinus	1.0- 3.0	1.42	1	0.674

Table 4.2. Range, median and standard deviation for day and night roost fidelity of *M. mystacinus* and *M. brandtii* from two study colonies for each species (n= 24, 12 and 12 respectively)

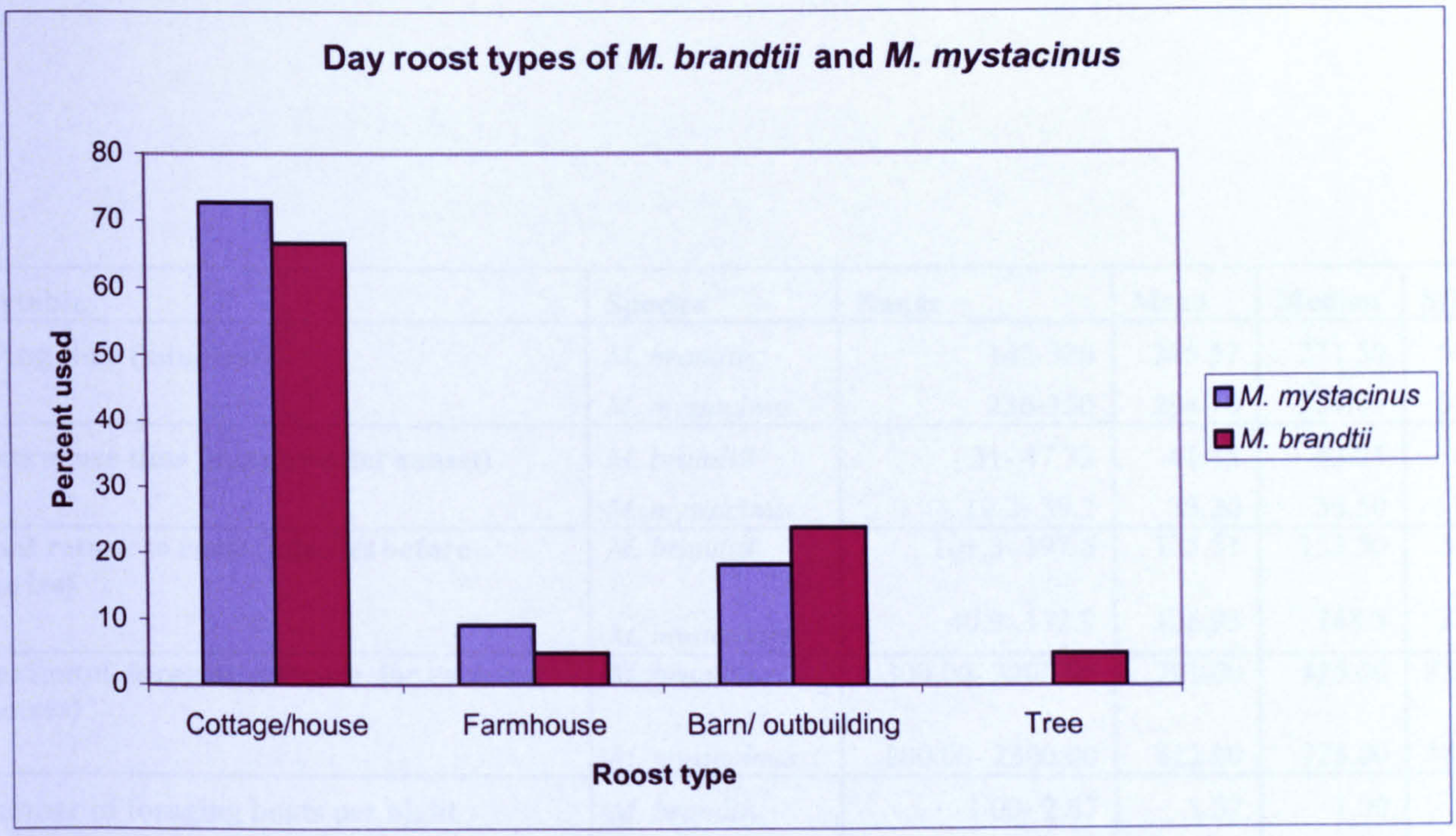

Figure 4.1. Comparison of day roost types for *M. mystacinus* and *M. brandtii* (n=24, 12 and 12 respectively)

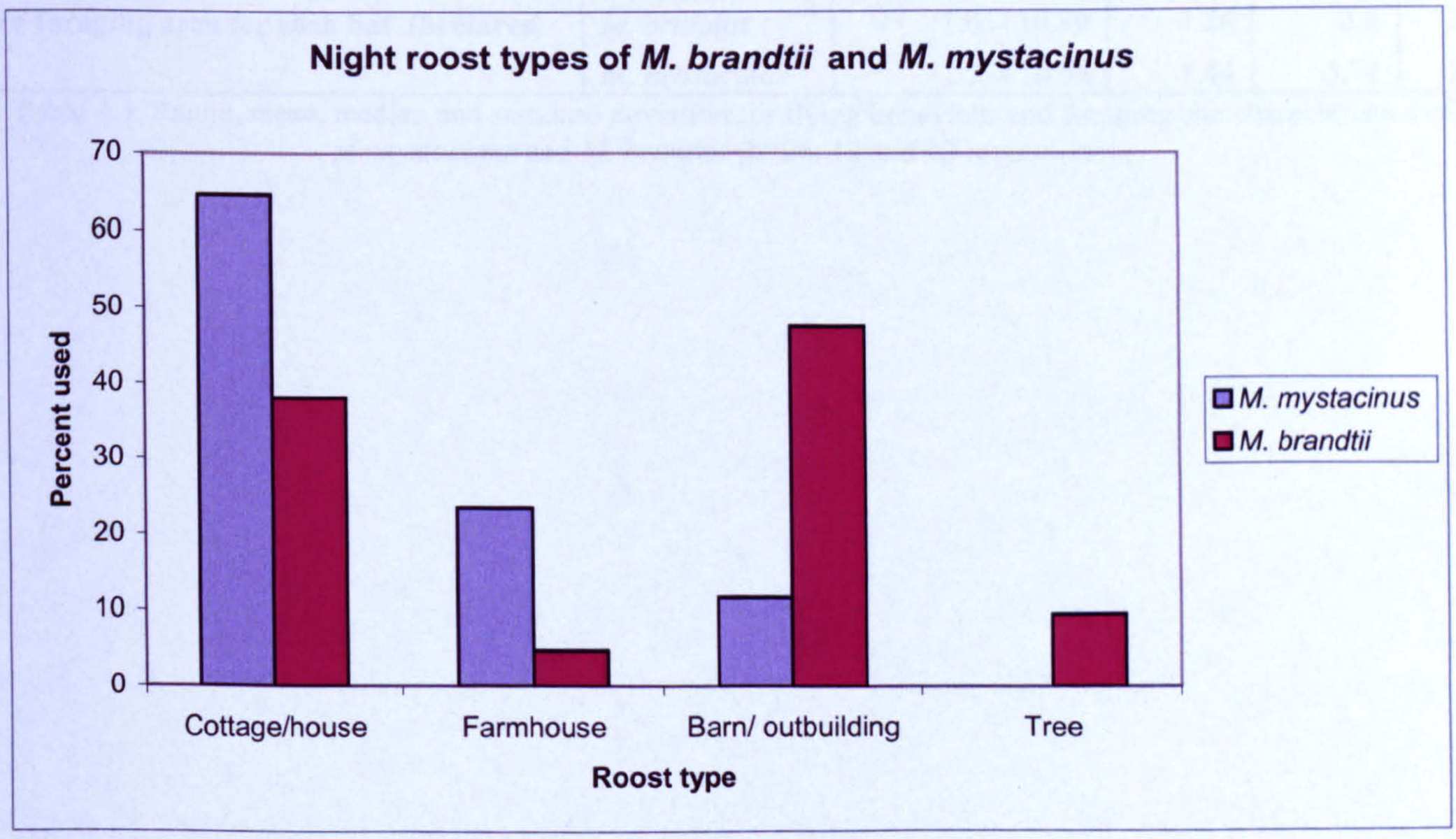

Figure 4.2. Comparison of night roost types for *M. mystacinus* and *M. brandtii* (n=24, 12 and 12 respectively)

Variable	Species	Range	Mean	Median	SD
Flying time (minutes)	*M. brandtii*	142-320	245.57	271.50	50.53
	M. mystacinus	236-350	294.70	304.00	35.31
Emergence time (minutes after sunset)	*M. brandtii*	31- 47.33	41.45	43.25	6.18
	M. mystacinus	19.2- 39.2	33.20	36.50	6.84
Final return to roost (minutes before sunrise)	*M. brandtii*	101.3- 197.5	135.51	133.50	32.21
	M. mystacinus	40.9- 172.9	126.93	148.5	38.10
Maximum foraging distance for each bat (metres)	*M. brandtii*	300.00- 3200.00	791.00	425.00	850.00
	M. mystacinus	200.00- 2300.00	812.00	725.00	586.00
Number of foraging bouts per night	*M. brandtii*	1.00- 2.67	1.57	1.00	0.48
	M. mystacinus	1.14- 2.33	1.55	1.00	0.35
Total MCP for each bat (hectares)	*M. brandtii*	5.40- 257.8	40.56	18.03	70.00
	M. mystacinus	13.20- 119.20	33.10	22.60	30.38
Number of foraging sites for each bat	*M. brandtii*	1.00- 4.00	1.50	2.00	0.65
	M. mystacinus	1.00- 3.00	1.25	1.00	1.13
Core foraging area for each bat (hectares)	*M. brandtii*	1.01- 10.89	4.26	3.6	2.84
	M. mystacinus	1.37- 10.66	5.44	5.71	2.96

Table 4.3. Range, mean, median and standard deviation for flying behaviour and foraging site characteristics of *M. mystacinus* and *M. brandtii* (n=24, 12 and 12 respectively)

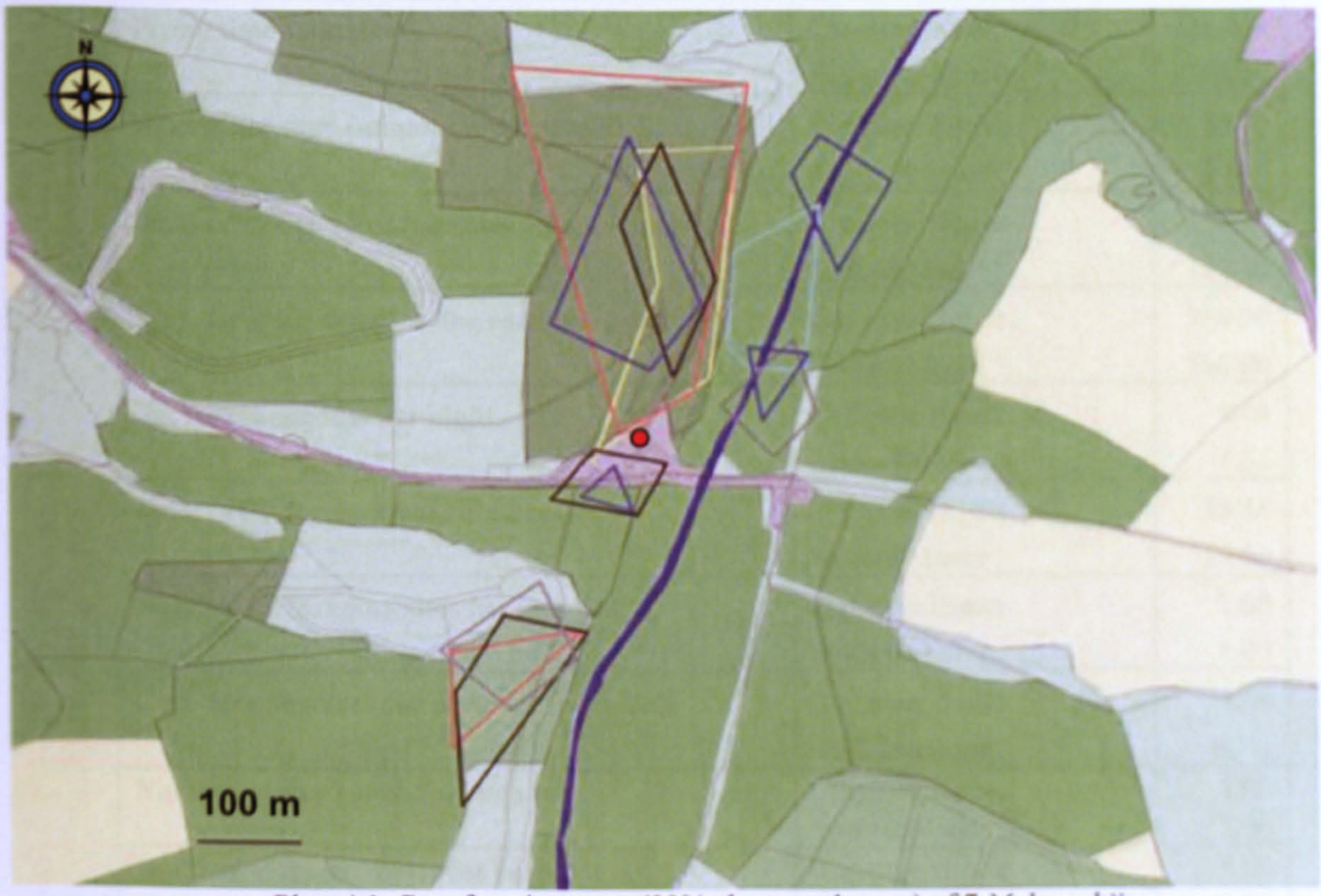

Plate 4.1. Core foraging areas (90% cluster polygons) of 7 *M. brandtii*
(the red dot indicates the main study colony)

Plate 4.2. Core foraging areas (90% cluster polygons) of 7 *M. mystacinus*
(the red dot indicates the main study colony)

Variable	Site	Median
Flying time (minutes)	Compton House (n=9)	295.00
	Stoford Manor (n=3)	294.50
Emergence time (minutes after sunset)	Compton House	37.00
	Stoford Manor	35.00
Final return to roost (minutes before sunrise)	Compton House	134.50
	Stoford Manor	169.00
Max. foraging distance for each bat (metres)	Compton House	750.00
	Stoford Manor	500.00
No. foraging bouts per night	Compton House	1.44
	Stoford Manor	1.67
Total MCP for each bat (hectares)	Compton House	25.61
	Stoford Manor	15.10
Number of foraging sites for each bat	Compton House	1.00
	Stoford Manor	1.00
Core foraging area for each bat (hectares)	Compton House	4.54
	Stoford Manor	6.82
Number of day roosts for each bat	Compton House	1.00
	Stoford Manor	1.00
Number of night roosts for each bat	Compton House	1.00
	Stoford Manor	1.00

Table 4.4. Flying behaviour, roost and foraging site characteristics of *M. mystacinus* (n=12)

Variable	Site	Median
Flying time (minutes)	Golden Mill (n=11)	219.00
	Church House (n=1)	219.00
Emergence time (minutes after sunset)	Golden Mill	41.50
	Church House	48.00
Final return to roost (minutes before sunrise)	Golden Mill	140.00
	Church House	115.00
Max. foraging distance for each bat (metres)	Golden Mill	450.00
	Church House	300.00
No. foraging bouts per night	Golden Mill	1.44
	Church House	1.67
Total MCP for each bat (hectares)	Golden Mill	18.40
	Church House	8.11
Number of foraging sites for each bat	Golden Mill	2.55
	Church House	2.00
Core foraging area for each bat (hectares)	Golden Mill	4.03
	Church House	4.03
Number of day roosts for each bat	Golden Mill	2.00
	Church House	1.00
Number of night roosts for each bat	Golden Mill	2.00
	Church House	1.00

Table 4.5. Flying behaviour, roost and foraging site characteristics of *M. brandtii* (n=12)

5. DIET

ABSTRACT

Similarities in echolocation call structure and morphology suggest that the foraging behaviour and diets of the cryptic species *M. mystacinus* and *M. brandtii* overlap. However, in order for animal species to coexist sympatrically, it is expected that they will occupy different ecological niches. Diet is an important dimension of the ecological niche and studies on the foraging ecology of cryptic species show that they often have different diets (e.g. Arlettaz 1996, Barlow 1997).

By collecting droppings from maternity colonies and carrying out faecal analysis I found significant differences between the diet of *M. mystacinus* and *M. brandtii* and also significant differences between the two *M. brandtii* study colonies. The faecal analysis showed that *M. mystacinus* and *M. brandtii* have a broad diet (Simpson index 0.140 and 0.088 respectively), comprised mostly of Diptera and Lepidoptera. A proportion of their prey is gleaned from diurnal prey groups such as Brachycera and Cyclorrhapha and non-flying arthropods such as Araneida. Both species show seasonal differences in their dietary diversity and composition. The life histories of some of the insect taxa in their diets support the difference in habitat use which has been found for the species (e.g. Taake 1984, Chapter 3) in that *M. mystacinus* forage in grassland, often grazed pasture surrounded by hedgerows, while *M. brandtii* is more reliant on woodland. However, insect families often have varied life histories and many insect taxa are found in a range of habitats so further inferences of habitat use could not be drawn from the results of the dietary study.

5.1. INTRODUCTION

In order for animal species to coexist sympatrically, it is expected that they will occupy different ecological niches (Hutchinson 1957). The similarities in wing morphology and echolocation call structure of the cryptic bat species *M. mystacinus* and *M. brandtii* suggest that their foraging habitats and diets may overlap, as bat species that are morphologically similar are expected to show little niche separation (e.g. Findley and Black 1983, Fenton 1990). However, a number of studies have shown that cryptic species forage in different habitats and have different diets (e.g. Herd and Fenton 1983, Saunders and Barclay 1992, Arlettaz 1996, Barlow 1997). Unlike previously studied cryptic bat species, *M. mystacinus* and *M. brandtii* have different evolutionary histories (Ruedi and Mayer 2001) and therefore provide an excellent opportunity for studying the mechanisms that permit resource partitioning.

Radiotracking studies show differences in habitat use between *M. mystacinus* and *M. brandtii*. While *M. mystacinus* forage mostly in different types of grassland (especially grazed pasture) surrounded by hedgerows, *M. brandtii* is more reliant on woodland (Chapter 3). Taake (1984) found similar results in a survey of habitat types around maternity colonies in Germany. The most common form of resource partitioning however, is by food specialisation, in which different bat species prey on insects of different taxonomic or size groups (e.g. Black 1974, Husar 1976, Fenton et al. 1977, Gaisler 1979, Hickey et al. 1996, Zhang et al. 2004), but temporal partitioning has also been reported (e.g. Kunz 1973, 1974, Reith 1980). Dietary analysis of insectivorous bats includes quantification of food components (e.g. Arlettaz 1996, Whitaker 1988), often including an assessment of whether evidence exists for prey selection (e.g. Swift and Racey 1983, Pereira et al. 2002). Previous studies have also examined whether diet varies with sex (e.g. Kunz 1974, Swift and Racey 1983, Leelapaibul et al. 2005), season (e.g. Pereira et al. 2002, Brack et al. 2006, Ma et al. 2006) and age of the bats (e.g. Carter et al. 1998, Hamilton and Barclay 1998) and whether differences occur between different populations of bats (e.g. Swift and Racey 1983, Johnston and Fenton 2001). Of particular interest however, is how dietary resources may be partitioned among sympatric species (e.g. Warner 1985, Saunders and Barclay 1992, Arlettaz 1996, Hickey et al. 1996, Barlow 1997).

Several dietary studies of *M. mystacinus* have been published with only one study published on the diet of *M. brandtii* so comparisons between their diets is poorly documented. A

German study by Taake (1992, 1993) compared the diets of the two species. He found the bats to have similar diets, mostly comprised of Lepidoptera and Diptera (Taake 1992, 1993). Robinson and Stebbings (1992) point out however, that Lepidoptera is often over-represented in dietary studies of bats. Taake (1992, 1993) also suggests that both species glean some of their prey due to the frequent presence of Araneida in their faeces. Taake (1992, 1993) concludes that the diet of the two species is similar also down to family level.

The aim of this study was to investigate differences in diet between *M. mystacinus* and *M. brandtii* because diet is an important dimension of the ecological niche.

5.2. METHODS

5.2.1. Choice of method

Due to the bats' conservation status and elusive behaviour only faecal analysis was considered a feasible option for diet analysis. Faecal analysis is accepted as a satisfactory technique for determining the diet of bats and is believed to be a reasonably accurate method (Kunz and Whitaker 1983), although differential digestion may cause some bias due to over or underestimation of certain items (Rabinowitz and Tuttle 1982). While small soft-bodied insects may be digested beyond recognition, large, chitinous prey will remain identifiable (Belwood and Fenton 1976). Kunz and Whitaker (1983) demonstrated that faecal analysis yields reasonable estimates of food eaten, particularly with commoner prey categories, however some authors point out that differences are seldom detectable at family and genus level (e.g. Ross 1967). The taxonomic level to which insect remains from faecal pellets can be determined depend much upon the species of insect digested, the amount of mastication and whether or not identifiable fragments such as legs, antennae or wings are discarded prior to ingestion (Ross 1967, Robinson and Fenton 1993). However, faecal analysis has been used successfully in a great number of dietary studies on various bat species (e.g. Swift and Racey 1983, Arlettaz 1996, Fenton et al. 1997, Zhang et al. 2004).

Any quantitative analysis must be treated with great caution as the results may be inaccurate and misleading because of the many inherent biases discussed above (Robinson and Stebbings 1993). The amount of remains per pellet depends upon mastication, and mastication is usually

greater for smaller species. Further bias may result from bats feeding continuously at feeding territories for several hours before returning to their roosts. During this time, rapid, in-flight digestion and defecation occur and consequently the relatively few insects caught en route back to the roost may occur in inordinately large numbers in faecal pellets collected from the roost (Rabinowitz and Tuttle 1982). *M. mystacinus* and *M. brandtii* usually forage within 2.3 and 3.2 km of the roost respectively and often night roost for several hours (Chapter 4) so this potential problem is therefore of less importance with these species. Additionally, when collecting droppings from bat roosts there is a chance that many of the droppings will contain prey caught in the latter part of the night. However, the radiotracked *M. mystacinus* and *M. brandtii* from the sampling sites had several feeding bouts each night (Chapter 4), which should reduce this bias.

5.2.2. Study sites

One primary and one secondary maternity colony were used for faecal collection for each species. All maternity colonies are in privately owned old, stone houses located in south west England. The colonies are surrounded by habitats of grassland (improved, semi-improved and semi-natural), woodland (mixed, deciduous and coniferous), amenity areas, built up areas and arable land. For grid references and size of the colonies, refer to Table 3.1. In all four colonies the bats were roosting above the main roof beam in the attic and there was no evidence of other species being present in the roosts.

5.2.3. Sampling methods

Faecal samples were collected by placing plastic sheets on the attic floor below where the bats were roosting. The plastic sheets were emptied monthly (except from the colony in Compton House, where it was emptied bimonthly). The two *M. mystacinus* colonies were sampled in 2003; while the two *M. brandtii* colonies were sampled in 2004, for sampling dates refer to Box 5.1. The droppings were stored in sealed petri-dishes.

Colony	Sampling dates
Compton House	08.05.03- 22.05.03- 04.06.03- 18.06.03- 01.07.03- 14.07.03
	28.07.03- 10.08.03- 25.08.03- 08.09.03
Stoford Manor	13.06.03- 16.07.03- 21.08.03- 15.09.03
Golden Mill	20.05.04- 19.06.04- 21.07.04- 24.08.04
Church House	23.05.04- 19.06.04- 21.07.04- 24.08.04

Box 5.1. Faecal sampling dates for each colony of *M. mystacinus* and *M. brandtii*

5.2.4. Faecal analysis

Kunz and Whitaker (1988) state that a minimum of 15 droppings should be analysed from each sample. 16 droppings were chosen randomly from each sample collected bimonthly (i.e. the samples from the colony in Compton House) and 32 droppings were chosen randomly from each sample collected monthly (i.e. the samples from the three remaining colonies). Faecal analysis was carried out according to Shiel et al. (1997). Each dropping was softened by soaking it in water for a few minutes. A small amount of glycerine was then added and the dropping was teased apart in a petri dish under a binocular microscope (Kyowa, x 10.5-60 magnification). All identifiable items were removed and mounted in glycerine on a microscope slide. The prey remains in each dropping were then identified mostly to order, but also to family level wherever possible, using insect identification guides e.g. Chinery (1997) and Skidmore (1991), a guide to insect remains in bat droppings (McAney et al. 1991) and a reference collection of whole insects. Lepidoptera was scored only when a large number of scales were present because moth scales remain in the digestive tract for long periods (Whitaker 1988, Robinson and Stebbings 1993). However, such droppings always contained other identifiable lepidopteran parts such as legs or antennae. Remains of the families Chironomidae and Ceratopogonidae could not be separated (Sullivan et al. 1993); hence these two prey families were considered as one prey group. Presence of mites, ticks and fleas in the droppings were scored, but have not been included in the statistical analysis because they were probably not an intentional part of the diet. The remains of fleas probably derived from grooming, but as all fleas were intact they may not have been eaten, but simply adhered to the droppings. The mites may also have been indigested during grooming. On the other hand, they could have been eaten along with other arthropods, as it is well known that e.g. beetles, especially the Scarabiidea, carry a variety of forms (Hyatt 1990).

5.2.5. Statistical analysis

A total of 225 *M. mystacinus* droppings and 196 *M. brandtii* droppings were analysed. The analysis yielded 757 arthropod fragments for *M. mystacinus*, where 9.7% remained unidentified, and 681 fragments for *M. brandtii* where 7.3% remained unidentified. The data was scored as percent items, which is defined as the number of items of each prey group as a percentage of the total number of identified remains of all prey groups in that sample, as suggested by Vaughan (1997). Vaughan also suggests using percent volume i.e. the estimated volume of the prey groups in a sample. Percent volume was not estimated in this study because the estimation was considered too inaccurate as the droppings mainly consist of very small, unidentifiable fragments resulting in prey taxa with many easily identifiable fragments appearing more prominent that they do in reality (Robinson and Stebbings 1993).

The difference in total diet between sites and species was analysed using Multiway Contingency tables on the raw data (Zar 1984) with a significance level of 5%.

Simpson's diversity index was used for comparison of dietary diversity between various groupings of bats. The Simpson index (D) is calculated by using the formula: $D= \Sigma pi^2$, where *pi* is the proportion of the *i*th class in the diet of a given colony. The Simpson index is defined as the probability of two individuals in a random sample being in the same category. The index is scored from 0-1, with zero being the most diverse. The Simpson index is a dominance measure and is weighted towards the most abundant species, not towards species richness (Magurran 1988). It is non-parametric and uses no underlying assumption about the shape of the underlying species abundance distribution.

5.3. RESULTS

5.3.1. Differences in total diet between species

Table 5.1 shows the dietary composition of *M. mystacinus* and *M. brandtii* during 2003 and 2004, the results are combined from the two colonies for each species. Anisopodidae made up almost a third of the diet for *M. mystacinus*. Lepidoptera made up 17%, while Scathophagidae and Calliphoridae made up just below 10% of the diet. Anisopodidae is a much less important component of the diet for *M. brandtii* and made up only 16%. Lepidoptera made up 12%, while Aranea, Hemerobiidae and Ichneumonidae each made up just above or below 10% of

the diet. *M. mystacinus* therefore seem to eat more Cyclorrhapha flies than *M. brandtii* and less Aranea and ichneumonids. Subsequent analysis performed after a multiway chi-square analysis showed that there were significant differences between the diet of *M. mystacinus* and *M. brandtii* (X^2= 95.4592, d.f. 20, P<0.001).

22.3% and 22.57% of the prey for *M. mystacinus* and *M. brandtii* respectively belong to insect taxa which are diurnal or rarely fly (Brachycera, Syrphidae, Cyclorrapha, Aranea and Aphidoidea), indicating a gleaning habit. Insect taxa with aquatic larvae (Chironomidae/Ceratopogonidae, Culicidae, Tipulidae, Psychodidae and Syrphidae) comprise 11.3% of the diet of *M. mystacinus* and 11.6% of the diet of *M. brandtii.* Insect taxa with adults living in close proximity to water (Hemerobiidae, Syrphidae, Culicidae, Chironomidae, Psychodidae, and Empididae) on the other hand comprise 14.1% of the diet of *M. mystacinus* and 20.5% of the diet of *M. brandtii. M. brandtii* has a larger proportion of insects taxa associated with woodland in the diet (Hemerobiidae, Ichneumonidae, Tipulidae, Rhagionidae and Syrphidae) than *M. mystacinus* (20.2% and 11.8% respectively); while *M. mystacinus* is more reliant on insects associated with pasture (Carabiidae, Scarabiidae and Scathophagidae) than *M. brandtii* (13.4% and 7.7% respectively).

5.3.2. Differences in diet between *M. mystacinus* colonies

The dietary composition of the two *M. mystacinus* colonies in 2003 can be seen in Table 5.2. At Compton House, Anisopodidae made up almost a quarter of the bats' diet. Scathophagidae, Calliphoridae and Lepidoptera each made up just below or above 10% of the diet. For the *M. mystacinus* colony at Stoford Manor Anisopodidae again made up about a quarter of the diet. Lepidoptera made up just over 20% of the diet. The bats at Stoford Manor therefore seem to eat more flies of the suborder Cyclorrhapha and more Lepidoptera compared to the bats at Compton Greenfield. However, a multiway chi-square analysis showed no significant differences in diet the between the two *M. mystacinus* colonies (X^2= 23.6026, d.f. 18, 0.25<P<0.10).

5.3.3. Differences in diet between *M. brandtii* colonies

Table 5.3 shows the dietary composition of the two *M. brandtii* colonies in 2004. Anisopodidae, Hemerobiidae, Psychodidae, Calliphoridae, Lepidoptera, Ichneumonidae and Aranea each made up just above or below 10% of the bats' diet in the colony at Golden Mill. For the *M. brandtii* colony at Church House, Anisopodidae made up almost a quarter of the

diet. Lepidoptera made up 14%. Hemerobiidae and Aranea each made up just below 10% of the diet. The bats at Golden Mill therefore seem to eat more Psychodidae, Calliphoridae and Ichneumonidae and less Anisopodidae and Lepidoptera than the bats at Church House. Subsequent analysis performed after a multiway chi-square analysis showed that there were significant differences in diet between the two *M. brandtii* colonies (X^2= 49.938, d.f. 17, P<0.001).

5.3.4. Differences in dietary diversity

There is little difference between the Simpson indices of *M. mystacinus* and *M. brandtii*; both species have a low Simpson index of 0.140 and 0.088 respectively indicating that both species have a broad diet, with *M. brandtii* having a slightly broader diet than *M. mystacinus*. Differences between the Simpson indices between sites are also small with the *M. mystacinus* colonies having Simpson indices of 0.082 and 0.086, while the *M. brandtii* colonies have Simpson indices of 0.124 and 0.085.

5.3.5. Seasonal differences in diet

Figure 5.1 shows changes in dietary diversity over time for both *M. mystacinus* colonies combined, measured by the Simpson index. The diet is most diverse in May, July and August, but note that these are only minor fluctuations. Figure 5.2 shows a similar graph for *M. brandtii* where the dietary diversity fluctuates more throughout the season than the dietary diversity of *M. mystacinus* and is at its most diverse in June and September. This indicates that the importance of different taxa in the diet varies throughout the season.

Changes throughout 2003 in items making up 9% or more of the total diet for both *M. mystacinus* colonies combined are shown in Figure 5.3. Calliphoridae fluctuates little throughout the season while Anisopodidae on the other hand decreases in importance from about 30% to 20% in July. Cyclorrapha increases in early June from about 5% to about 15%. Lepidoptera increases throughout the season from 10% at the start of the season to almost 25% at the end of the season. There seem to be no general trend in seasonal differences in the importance of different prey taxa in the diet of *M. mystacinus*.

Figure 5.4 shows similar data for the two study colonies for *M. brandtii* during 2004. The graph indicates that Hemerobiidae shows only minor changes throughout the season. Ichneumonidae decreases throughout the season from almost 15% at the start of the season to

5% at the end of the season. Lepidoptera shows an increase in July from 10% to 15%. Anisopodidae increases from 15% to almost 25% from May to August, but decreases in September to just over 5%. Similarly to *M. mystacinus*, there seem to be no general trend in seasonal differences in dietary importance of different prey taxa for *M. brandtii*.

5.4. DISCUSSION

5.4.1. Foraging behaviour and habitat use

The results provide some evidence that both species glean at least an appreciable fraction of their prey from surfaces such as leaves and tree trunks, in the form of diurnal insects such as Brachycera, Syrphidae and Calliphoridae flies (Lewis and Taylor 1964), non-flying arthropods (Aranea) and Aphidoidea. These taxa comprised 22.3% and 22.57% of the diet of *M. mystacinus* and *M. brandtii* respectively. However, Aranea may have been caught in the air while suspended on silken threads, referred to as ballooning. Nearly 30% of the approximately 540 species of Microchiroptera, which eat mainly animal protein, might be expected to obtain their prey from gleaning, i.e. capturing prey on the ground, off tree bark, cliff faces or from foliage. This figure is based on their external morphology (e.g. Handley 1959, Wilson 1973, Black 1974, Findley 1976, Bell 1980).

Another component of the diet (11.3% of the diet of *M. mystacinus* and 11.6% of the diet of *M. brandtii*) consists of insects with aquatic larvae such as the dipteran families Chironomidae/Ceratopogonidae, Culicidae, Tipulidae, Psychodidae and Syrphidae. Several of the taxa eaten by the bats (Hemerobiidae, Syrphidae, Culicidae, Chironomidae, Psychodidae, and Empididae) also have adults living in close proximity to water (total of 14.1% of the diet of *M. mystacinus* and 20.5% of the diet of *M. brandtii)*. Therefore, presumably a significant proportion of the adult insects were caught near water. Hemerobiidae, Ichneumonidae, Tipulidae, Rhagionidae and Syrphidae all have members which are found in woodland or woodland edge. These taxa comprised 11.8% of the diet of *M. mystacinus* and 20.2% of the diet of *M. brandtii.* On the other hand, many of the prey families have members associated with pasture, especially pasture with dung. These families include Carabiidae, Scarabiidae and Scathophagidae (comprising 13.4% of the diet of *M. mystacinus* and 7.7% of the diet of *M. brandtii*). Additionally, many dipteran families have members which occasionally visit dung to breed or suck moisture. The dietary composition of *M. brandtii* and *M. mystacinus*

therefore suggests that both species catch prey near water, in woodland and in pasture. *M. brandtii* however, eat more insects associated with woodland, while *M. mystacinus* eat more insects associated with pasture. Because many insect families have members with a great variety in their life histories, it is difficult to draw further inferences about the bats' habitat use from their diet. However, the inferences drawn still correspond well with previous data published on the habitat use of *M. brandtii* and *M. mystacinus* (e.g. Taake 1984) and the results presented in Chapter 3; with *M. mystacinus* foraging in different types of grassland (especially grazed pasture surrounded by hedgerows) and *M. brandtii* being more reliant on woodland.

Herd and Fenton (1983) found in a dietary study of *M. lucifugus* and *M. yumanensis* that the bats' differences in diet probably reflected differences in foraging habitats. Harbusch and Racey (2004) found that foraging habitats were chosen according to the absolute densities and diversity of preferred prey taxa. Selectivity in terms of prey is likely to a large degree to result from selection of a particular foraging habitat, rather than selection of a particular type of insect, and once the habitat is chosen, the bats may simply feed on whatever appropriate-size insect is most abundant (Aldridge and Rautenbach 1987, Brigham 1990, Barclay and Brigham 1994, Whitaker 1995, Kurta and Whitaker 1998). It has also been shown that bats may forage during different time periods (Kunz 1973). This could lead to dietary differences between species, however, the results presented in Chapter 4 show that there is little difference in the timing of their foraging behaviour; habitat use on the other hand, does differ between the two species (Chapter 3) and may therefore lead to the difference in diet between the two species. Refer to section 6.1 for a discussion on the conservation of the prey and foraging habitats of *M. brandtii* and *M. mystacinus*.

5.4.2. Geographical and intra-specific variation

A multiway chi square analysis showed dietary differences between the two *M. brandtii* sites. Rindle and Zahn (1997) found differences of more than 50% between *M. mystacinus* colonies in a dietary study; they suggested that differences in habitat use may explain the differences in diet. However, as discussed in Chapter 3, my study found only minor differences in habitat use and foraging behaviour between the two *M. mystacinus* colonies and the two *M. brandtii* colonies studied. Other studies have also found differences in dietary composition at different sites for the same species. Johnston and Fenton (2001) studied the diet of *Antrozous pallidus* at two locations in California. They found that variation in diets between two locations

reflected prey availability and individual foraging behaviour. Sample and Whitmore (1993) found significant differences among *Plecotus townsendii virginanus* from three different maternity caves in eastern West Virginia and suggested that variation in the amount of Coleoptera consumed at the different caves might be related to the proximity of open fields, whereas variation in the amount of Diptera and Hymenoptera reflect different abundance of these orders. Terrestrial insects (Lepidoptera and Coleoptera) dominated the diet of *Myotis sodalis* in more southern states of the United States while in Michigan the bats mostly consumed insects associated with aquatic habitats (e.g. Trichoptera and Diptera) (Kurta and Whitaker 1998). There is currently no accurate method of assessing prey availability and prey abundance for bats that glean much of their prey, they were therefore not investigated in my study. It is therefore impossible to discuss whether differences in prey abundance or availability account for any of the dietary differences between the two species or between the two *M. brandtii* colonies.

Some studies indicate that diet varies from generalised to specialised for the same species of bat. My study showed that *M. mystacinus* and *M. brandtii* have a Simpson index indicating that both species have a broad diet, especially *M. brandtii*, and that there is little difference between the dietary breadth of the colonies studied for each species. Long-term studies of prey selection by some bats indicate that intra-specific diet variability may be as great as apparent inter-specific differences (e.g. Kunz 1974, Belwood and Fenton 1976, Anthony and Kunz 1977). Isolated examples of extreme prey selectivity have been revealed by short-term studies (Buchler 1976, Whitaker and Black 1976), this may be the result of opportunistic responses to mono-specific patches or swarms of prey rather than of selective feeding behaviour (Kunz 1973, Fenton and Morris 1976, Gould 1978, Vaughan 1980). Furthermore some species show a high degree of variability in their foraging behaviour (Vaughan 1976, Fenton and Bell 1979). Little can be concluded about the feeding habits of bats without consideration of the patterns of habitat use of different species in a community, and the spatial and temporal distribution of the food base. This may explain the results from a study of *M. mystacinus* at the same sampling site (Compton Greenfield) that was used in my study where Hollyfield (1993) found a Simpson index of 0.269, suggesting that the bats' diet was much less diverse than my results. This result may also be explained by differences in methods, habitat change or differences in weather however.

Husar (1976) found that the cryptic species *Myotis evotis* and *M. auriculus* had similar diets in areas of allopatry and different diets in areas of sympatry. While this may corroborate with the competition hypothesis, it may also reflect geographical or temporal variation because the sampling was carried out in different years. Arlettaz (1997) on the other hand, found that *Myotis myotis* ate terrestrial insects e.g. carabid beetles while *M. blythii* ate grass-dwelling insects, mostly bush crickets. Diet and niche breaths were similar in both areas of allopatry and sympatry. No colonies of *M. mystacinus* and *M. brandtii* were found in the same geographical area during my study so they could therefore not be studied in sympatry (except at swarming sites). Samples were in fact collected from swarming bats at two sites, but the sample sizes at the end of the project were too small for analysis. However, it is reasonable to assume that areas of allopatry exceed areas of sympatry (Mayr 1963). It should also be noted however that the effects of competition may not be due to just one single species.

5.4.3. Seasonal differences

Seasonal differences were found in both dietary breath and dietary composition for *M. mystacinus* and *M. brandtii* in all colonies. Arlettaz (1997) found seasonal variation in the diets of *Myotis myotis* and *M. blythii* and Pereira et al. (2002) also reported seasonal variation in the dietary composition and prey selection of *M. myotis* and also found that prey was far more abundant in the spring than in the summer. *Rhinolophus ferrumequinum* had a more diverse diet in spring and autumn when food was scarce; there were also differences in food composition (Jones 1990). Hollyfield (1993) found that the diet of *M. mystacinus* was most diverse in May and least diverse in June and July at the same sampling site (Compton Greenfield) used in my study. These results are similar to the results from my study.

There is yet no satisfactory general method of assessing prey availability for insectivorous bats which are principally gleaners (Kunz 1988), it was therefore not attempted in my study and it is almost impossible to discuss prey selection without a knowledge of availability. However, Williams (1939), from extensive light trapping on arable land in England showed that peak numbers for most orders were in July, with markedly lower values in May and September. It is not unreasonable to infer that probably, in general, free-living insects are most abundant in England in midsummer (Shiel et al. 1991) and that the availability of different taxa varies throughout the season leading to the seasonal differences in bat diet. The seasonal differences may also be explained by a change in habitat use as reported by Nyholm (1965) who found *M. mystacinus* to change habitats throughout the season; it is difficult to

determine if the bats from my study colonies showed similar foraging behaviour since they were only radiotracked for a short period of time and due to the short life of the radiotags.

5.4.4. Comparisons with other dietary studies of *M. mystacinus* and *M. brandtii*

When looking at the diet of *M. brandtii* it is important to keep in mind that early records of hunting and feeding ecology may also refer to *M. mystacinus*. Only the studies carried out in Germany by Taake (1992, 1993) also investigate the diet of *M. brandtii*. Taake found that out of 22 animals examined (results from faecal analysis and expressed as % animals, total >100%), 91% had eaten Diptera. Many of the dipteran families were diurnal suggesting a gleaning habit, which is also supported by my results. Taake also reports that 59% of the animals examined had eaten Arachnida spp., which leads to a similar conclusion. 91% of the specimens examined had eaten unidentified Lepidoptera, but this group is generally thought to be over represented in dietary studies of bats (Robinson and Stebbings 1993). Taake's study also showed that although *M. brandtii* has been found to hunt close to water (Taake 1984); the species is not particularly reliant on aquatic insects.

Taake also reported that out of 22 *M. mystacinus* specimens examined (again results from faecal analysis and expressed as % animals, total> 100%), over 95% had eaten Diptera (Taake 1992, 1993). A Swiss study by Beck (1994-1995) supports these findings. Taake (1992, 1993) found lepidopteran remains in 77% of the animals. However, Hollyfield (1993) (results from faecal analysis and expressed as % number of animals) found that Lepidoptera had only been eaten by 35% of the animals, while Beck (results from faecal analysis and expressed as % occurrence) reported lepidopteran remains in only 14% of the animals. This suggests that lepidopteran remains may be over represented in the first study, a common problem in diet analysis of bats (Robinson and Stebbings 1993). Rindle and Zahn (1997) found that remains of Lepidoptera and Diptera were most frequently occurring in the faeces (found in more than 88% of all faecal pellets). Note that Rindle and Zahn again point out that remains of non-flying prey indicate a gleaning habit, which is also supported by the results from my study. Remains of the order Arachnida, which were found in 82% of the animals in Taake's study (1992, 1993), suggest the same. My results show a much lower amount of Araneida in the diets of both species, especially *M. mystacinus*, this may due to habitat differences. Rindle and Zahn (1997) found differences of 50% or more in the diet of *M. mystacinus* colonies and they suggest that the difference may be due to differences in habitat surrounding the colonies.

Note that it is difficult to compare results from other dietary studies of *M. mystacinus* and *M. brandtii* due to the different ways of expressing the results.

Taake found that the two species had very similar diets. My study however, found a significant difference in the dietary composition of *M. mystacinus* and *M. brandtii*, this difference between studies may be explained by differences in methods, the fact that *M. mystacinus* and *M. brandtii* are at the edge of their geographical range in the UK or by differences in habitat types around the bat colonies.

5.4.5. Diet in relation to morphology and competition

Combined data on bite forces and food hardness can both directly and indirectly limit dietary diversity in bats. Bit performance may potentially result in a decrease in trophic breath for some species through its effect on dietary specialisation (Aguirre et al. 2004). Freeman (1979, 1981) found correlations between skull and jaw morphology and Black's (1974) dietary groupings of bats. There are currently no data available on differences in skull morphology between *M. mystacinus* and *M. brandtii*, but it is likely that their skull and jaw morphologies are similar and should therefore not lead to any dietary differences. Their body sizes are also similar suggesting that they probably do not partition their prey by size although this was not looked into in this study due to the prey remains being very well masticated making any size estimations very difficult. The echolocation calls of the two species are also similar, they should therefore be able to catch similar prey and be able to forage in similar habitats (Jones et al. 2000). There is only a small difference in the wing morphologies of *M. mystacinus* and *M. brandtii*, with *M. brandtii* having a slightly higher aspect ratio, making it less manoeuvrable (Chapter 2). However, this difference was not statistically significant. Jones (1991) found similar results. Therefore, in both studies differences in wing morphologies are very small and probably do not explain why *M. brandtii* and *M. mystacinus* have different diets.

Because the number of insects in a particular area is almost too overwhelming to comprehend at times, it is difficult to determine whether competition for food between two or more species of bats, or between bats and birds is more than incidental. Great numbers of other available insects of similar size, life form, and habits occur in the same habitat, hence, theoretically, bats would be expected to have a much broader diet. It appears there are several causes for rejection of prey such as unattractive or offensive odour, foul taste, excessive water of other

fluids, too thick an exoskeleton or colour markings that have a deterrent psychological effect. However, a laboratory study by Nyholm (1965) showed that the bats (*M. mystacinus*) preferred Lepidoptera. The bats would eat nothing else if these were offered. The animals would also take midges, flies, crane flies, dragonflies, mayflies, beetles and even bed bugs. Nyholm concluded that his captive animals were not particularly selective about their food. This was with the exception of plant remains, which were offered, but none taken. Nyholm also noted individual differences in choice of foods, but found no difference between the preferences of young bats and adults under experimental conditions (Nyholm 1965).

There is conflicting evidence about whether coexisting species of bats eat different food. Some studies show that bat species foraging in the same areas consume the same food (Aldridge and Rautenbach 1987, Saunders and Barclay 1992), whereas other studies provide evidence of diet partitioning and specialisation in coexisting species (Black 1974, Rydell 1989). For some species, there is evidence of spatial and temporal (Kunz 1973, 1974, Reith 1980) partitioning of food. Roosting requirements may limit the geographic range of certain species (Humprey 1975), but there are few data suggesting that competition for roosts structures communities of bats (Findley 1993). It may be that insect prey is normally so abundant that competition between insectivorous organisms is rare and limited to infrequent periods of unfavourable environmental conditions (Warner 1985). However, some temperate zone bat species appear to allocate their resources (Jones 1965, Kunz 1973, Black 1974) by spatial or temporal partitioning (Jones 1965, Kunz 1973) or by the selection of prey type (Ross 1967, Black 1974, Hickey et al. 1996) or size (Zhang et al. 2004). Although such dietary specialisation may be a result of competition, direct evidence is lacking.

A dietary study of swarming bats was attempted in order to minimise possible bias caused by geographical variation or variation between years. Additionally, such a study has the advantage of the bats feeding in sympatry around these sites. However, the sample sizes at the end of the project were too small for analysis. Dietary studies at swarming sites should therefore be attempted in the future in addition to dietary studies of bats from a larger number of study sites in different geographical areas.

5.5. CONCLUSION

The morphological differences between *M. mystacinus* and *M. brandtii* are small and probably do not explain the dietary differences in this study. Differences in habitat use and the associated differences in insect abundance and composition in these habitats is a more reasonable explanation for dietary differences. The habitat use of *M. mystacinus* and *M. brandtii* is further discussed in Chapter 3.

Taxa	Latin name	Common name	% of total diet	
			M. mystacinus (n=757)	*M. brandtii* (n=681)
Order	**Dermaptera**	**Earwigs**	0.3	
Order	**Hemiptera**	**True bugs**		
Family	Delphacicae		0.2	
	Psylloidea			0.1
	Aphidoidea			2.8
Order	**Neuroptera**	**Lacewings etc.**		
Family	Hemerobiidae		5.6	9.6
Order	**Coleoptera**	**Beetles**		
Family	Unidentified Coleoptera		1.3	2.1
	Carabidae		0.2	0.1
	Scarabiidae		2.7	1.7
Order	**Diptera**	**True flies**		
Family	Unidentified Diptera		5.6	12.1
Suborder	Nematocera			
Family	Anisopodidae		27.3	16.0
	Tipulidae		3.5	1.0
	Psychodidae		2.3	5.8
	Culicidae		1.7	0.7
	Chironomidae		3.2	3.8
	Mycetophilidae		0.8	
	Scatopsidae		0.8	
	Cecidomyiidae		0.2	
Suborder	Brachycera			
Family	Rhagionidae			0.3
	Empididae		0.7	0.3
Suborder	Cyclorrhapha			
Family	Syrphidae		0.6	0.3
	Sphaeroceridae		1.0	
	Ephydridae			0.1
	Drosophilidae		0.2	
	Calliphoridae		8.3	5.2
	Scathophagidae		9.2	3.8
	Fanniidae			0.1
Order	**Lepidoptera**	**Butterflies and moths**	17.0	12.2
Order	**Trichoptera**	**Caddis flies**		
Family	Limnophilidae		0.2	0.3
	Hydropsychidae			2.7
Order	**Hymenoptera**	**Bees, ichneums etc.**		
Family	Ichneumonidae		2.1	9.0
	Chalcicoidea		1.0	0.4
	Proctotrupidae		0.2	
Order	**Araneida**	**Spiders**		
Family	Argasidae		0.8	0.1
	Acari		0.5	
	Aranea		2.3	9.3
Order	**Psocoptera**	**Booklice**		0.1
Order	**Ephemeroptera**	**Mayflies**		
Family	Siphlonuridae		0.2	

Table 5.1. Differences in total diet between *M. mystacinus* and *M. brandtii*, shown in % items

Taxa	Latin name	Common name	% of total diet	
			Compton House (n=465)	Stoford Manor (n=216)
Order	**Dermaptera**	**Earwigs**	0.5	
Order	**Hemiptera**	**True bugs**		
Family	Delphacicae			0.5
	Psylloidea			
	Aphidoidea			
Order	**Neuroptera**	**Lacewings etc.**		
Family	Hemerobiidae		5.0	5.7
Order	**Coleoptera**	**Beetles**		
Family	Unidentified Coleoptera		2.8	2.6
	Carabidae		0.2	
	Scarabiidae		3.0	2.0
Order	**Diptera**	**True flies**		
Family	Unidentified Diptera		9.8	8.5
Suborder	Nematocera			
Family	Anisopodidae		23.2	25.0
	Tipulidae		4.2	2.0
	Psychodidae		2.6	1.6
	Culicidae			3.1
	Chironomidae		3.5	4.7
	Mycetophilidae		0.7	1.0
	Scatopsidae		0.9	
	Cecidomyiidae		0.2	
Suborder	Brachycera			
Family	Rhagionidae			
	Empididae		1.2	2.5
Suborder	Cyclorrhapha			
Family	Syrphidae		0.5	0.8
	Sphaeroceridae			1.0
	Ephydridae			
	Drosophilidae			0.5
	Calliphoridae		10.3	7.3
	Scathophagidae		5.8	4.7
	Fanniidae			
Order	**Lepidoptera**	**Butterflies and moths**	15.0	22.0
Order	**Trichoptera**	**Caddis flies**		
Family	Limnophilidae		0.2	
	Hydropsychidae			
Order	**Hymenoptera**	**Bees, ichneums etc.**		
Family	Ichneumonidae		2.1	2.0
	Chalcicoidea		1.2	
	Proctotrupidae		0.2	
Order	**Araneida**	**Spiders**		
Family	Argasidae		0.8	0.5
	Acari		1.1	
	Aranea		2.8	1.0
Order	**Psocoptera**	**Booklice**		
Order	**Ephemeroptera**	**Mayflies**		
Family	Siphlonuridae		0.2	0.5

Table 5.2. Differences in total diet between the *M. mystacinus* colonies, shown in % items

Taxa	Latin name	Common name	% of total diet	
			Golden Mill (n= 524)	Church House (n=233)
Order	**Dermaptera**	**Earwigs**		
Order	**Hemiptera**	**True bugs**		
Family	Delphacicae			
	Psylloidea		0.2	
	Aphidoidea		2.7	3.2
Order	**Neuroptera**	**Lacewings etc.**		
Family	Hemerobiidae		9.4	9.9
Order	**Coleoptera**	**Beetles**		
Family	Unidentified Coleoptera		1.4	3.6
	Carabidae		0.2	
	Scarabiidae		1.6	1.8
Order	**Diptera**	**True flies**		
Family	Unidentified Diptera		11.7	13.0
Suborder	Nematocera			
Family	Anisopodidae		12.0	23.0
	Tipulidae		1.0	1.0
	Psychodidae		6.3	4.5
	Culicidae		0.4	1.4
	Chironomidae		4.4	5.0
	Mycetophilidae			
	Scatopsidae			
	Cecidomyiidae			
Suborder	Brachycera			
Family	Rhagionidae		0.4	
	Empididae		0.2	0.5
Suborder	Cyclorrhapha			
Family	Syrphidae		0.4	
	Sphaeroceridae			
	Ephydridae		0.2	
	Drosophilidae			
	Calliphoridae		7.4	0.5
	Scathophagidae		4.7	1.8
	Fanniidae			0.5
Order	**Lepidoptera**	**Butterflies and moths**	12.0	14.0
Order	**Trichoptera**	**Caddis flies**		
Family	Limnophilidae			0.9
	Hydropsychidae		2.7	1.9
Order	**Hymenoptera**	**Bees, ichneums etc.**		
Family	Ichneumonidae		12.0	3.0
	Chalcicoidea		0.4	0.5
	Proctotrupidae			
Order	**Araneida**	**Spiders**		
Family	Argasidae			0.5
	Acari			
	Aranea		8.1	9.5
Order	**Psocoptera**	**Booklice**	0.2	
Order	**Ephemeroptera**	**Mayflies**		
Family	Siphlonuridae			

Table 5.3. Differences in total diet between the *M. brandtii* colonies, shown in % items

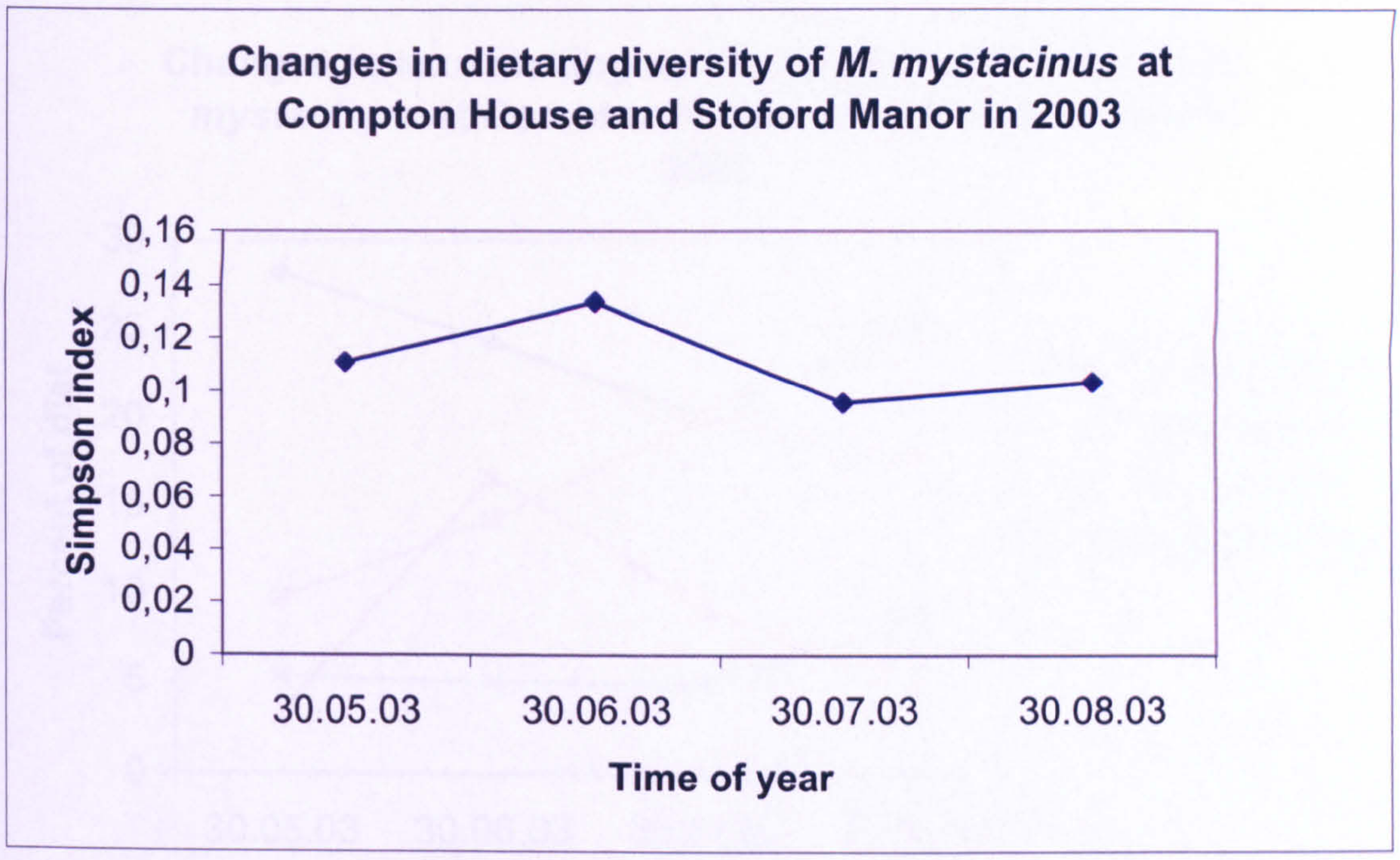

Figure 5.1. Changes in dietary diversity of *M. mystacinus* at Compton House and Stoford Manor from May to September 2003, measured by the Simpson index

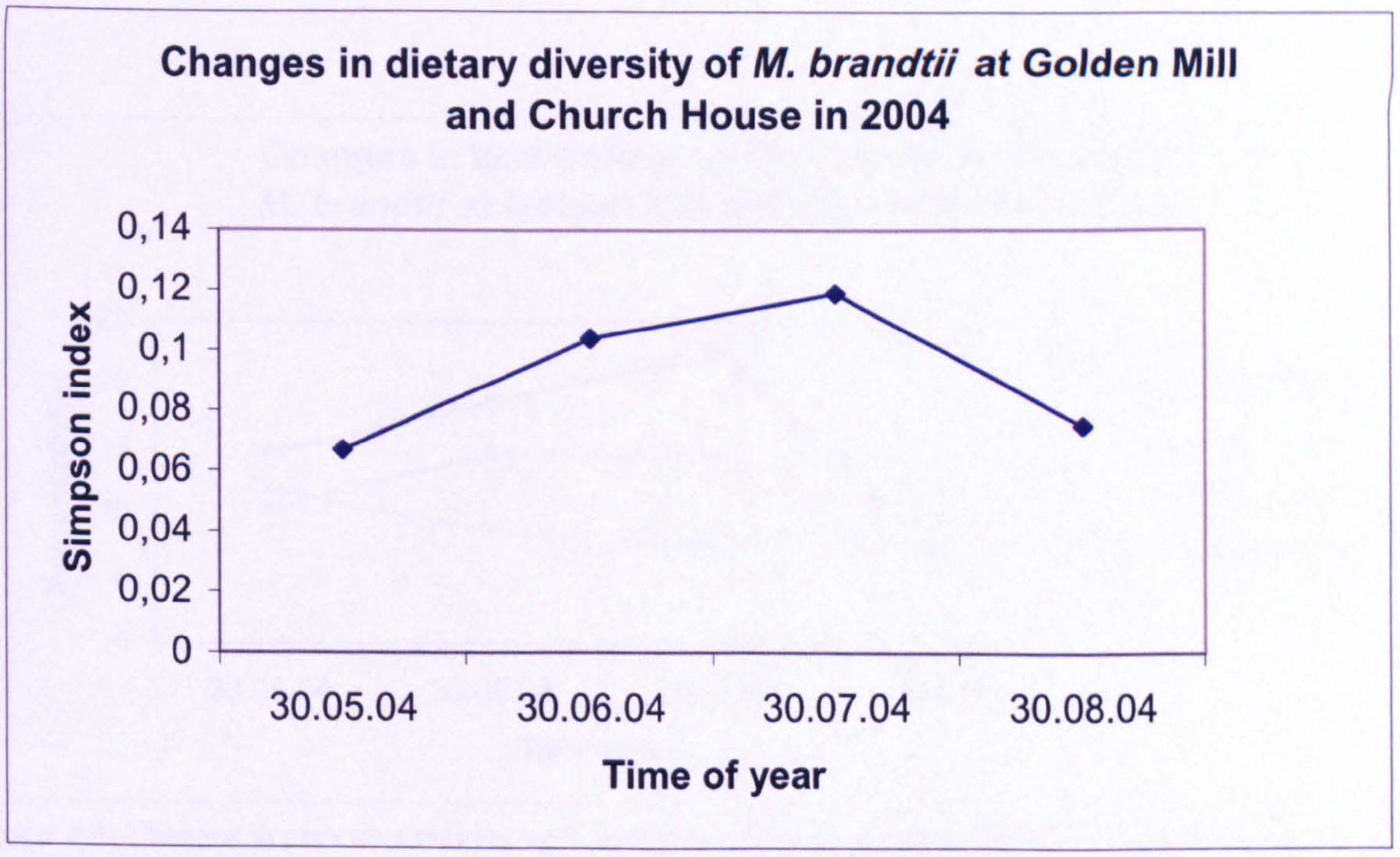

Figure 5.2. Changes in dietary diversity of *M. brandtii* at Golden Mill and Church House from May to September 2004, measured by the Simpson index

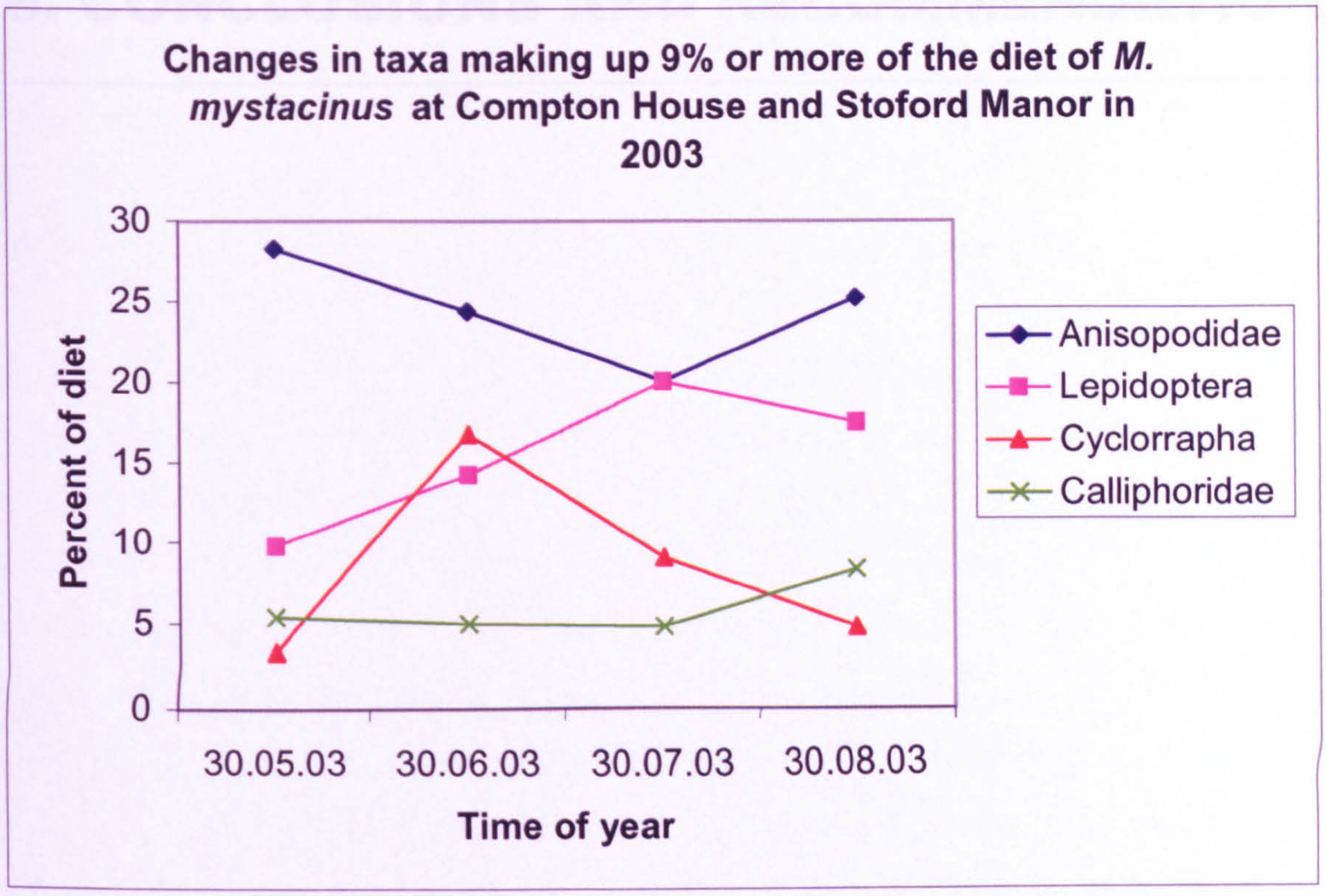

Figure 5.3. Changes in prey taxa making up 9% or more of the total diet of the *M. mystacinus* at Compton House and Stoford Manor between May and September 2003

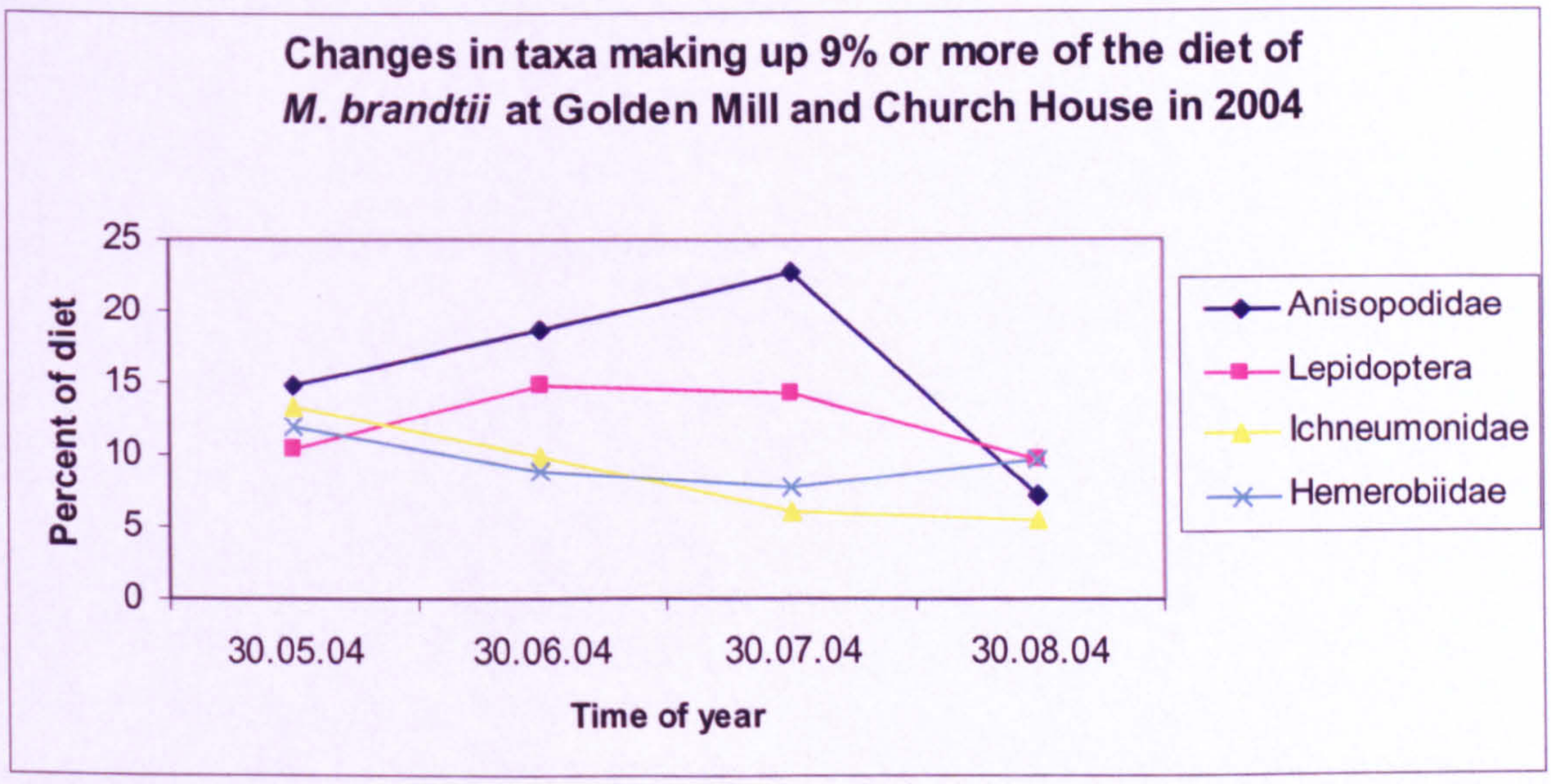

Figure 5.4. Changes in prey taxa making up 9% or more of the total diet of the *M. brandtii* at Golden Mill and Church House between May and September 2004

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

M. mystacinus and *M. brandtii* are almost identical in morphology and there is no single morphological feature which was tested in this study that showed no overlap between the species. However, by using a combination of the morphological features penis shape, tragus shape, upper jaw dentition, lower jaw dentition and thumb claw length it is possible to distinguish between the two species with 100% certainty.

Molecular studies have shown that *M. mystacinus* and *M. brandtii* have different evolutionary histories (Ruedi and Mayer 2001). Nevertheless, even with such similar morphologies the study showed that the two species differ in diet, foraging behaviour and habitat use. While *M. brandtii* was most reliant on woodland (especially coniferous woodland), *M. mystacinus* foraged in grassland, often pasture surrounded by hedgerows. Some habitat use studies on the continent have showed the species to have more similar habitat use than in my study, although these studies have used other methods such as acoustic and habitat surveys around maternity colonies (e.g. Taake 1984, Ahlén and DeJong 1996, Ahlén 1997, van der Coelen and Verheggen 1997, Vaughan et al. 1997, Buckley 2004). Differences in habitat use may be due to competition. Studies of the two species in allopatry and in sympatry are needed to establish whether there is evidence of inter-specific competition. However, if no competition is inferred from such studies, it is important to note that the absence of current competition does not imply that it has not been important as a structuring force in the past. Ecological differences may be due to what is often referred to as the "ghost of competition past", where selection in the past led to niche differentiation in order to avoid competition. It seems likely that selection has favoured the avoidance of competition through differences in habitat use. However, it is possible that the results are due to the study area being on the edge of the distribution range for these species. Climate is strongly correlated with latitude with higher amount of rainfall, reduced ambient temperatures, shorter night length and higher winds, inhibiting the foraging activity of bats and the abundance of insects (Nicholls and Racey 2006a). If competition existed, it would be heightened at the northern edge of their range leading to an increase in habitat partitioning, which again lead to differences in foraging behaviour and diet and may also explain differences between other habitat use studies of the two species.

Night roost fidelity, time of last return, number of foraging bouts per night, maximum foraging distance and MCP size were similar for the two species. However, *M. mystacinus* emerged earlier than *M. brandtii*, had longer flying times and changed day roosts less frequently. One particularly interesting result was that *M. brandtii* used more foraging areas than *M. mystacinus*, which usually only had one core area. *M. brandtii* also shared foraging areas with other conspecifics, unlike *M. mystacinus* in this study (although only a small number of bats were radiotracked at any one time). Differences between numbers of foraging sites and foraging site fidelity may be due to differences in food predictability and availability, with species having less foraging sites and showing greater foraging site fidelity foraging on predictable resources (Nicholls and Racey 2006a). Differences between individual and group foraging on the other hand, may be due to a long duration of food availability and an even distribution of food patches leading to individual foraging, while clumped, ephemeral food can select for group foraging and information transfer (Nicholls and Racey 2006a). The observation that *M. brandtii* used more foraging areas may therefore relate to their food resources being more unpredictably distributed, and group hunting may facilitate the location of such ephemeral food patches. Another interesting result was *M. mystacinus* having a longer average flying time than *M. brandtii*. One would expect *M. brandtii* which have a higher number of foraging areas and forage in habitats thought of as being of inferior quality as foraging habitat for bats, to forage for a longer period of time. Hence, coniferous woodland may be more important as foraging habitat for bats than previously expected.

Diet was significantly different between the two species. Although both species had broad diets comprised mostly of Diptera and Lepidoptera, about 20% of their prey was comprised of insect taxa which are diurnal or rarely fly, suggesting a gleaning habit. Both species show seasonal differences in dietary diversity and composition. Differences in diet are probably reflected by the differences in habitat use as different habitats contain different insect taxa. It is therefore likely that differences in foraging behaviour and habitat use are mainly reflections of the differences in habitat use. The results from the dietary support to some extent the habitat use results, but note that insect families of often have different life histories and exploit a range of habitats, further inferences about habitat use can therefore not be drawn from this study.

The two other cryptic species pairs which have been intensively studied; *M. myotis* and *M. blythii* (Audet 1990, Arlettaz 1996, Arlettaz et al. 1997, Arlettaz 1999) and *Pipistrellus*

pipistrellus and *P. pygmaeus* (Swift 1980, Swift and Racey 1983, Racey and Swift 1985, Avery 1986, Barlow 1997, Barlow et al. 1997, Davidson- Watts et al. 2006, Davidson- Watts and Jones 2006, Nicholls and Racey 2006a, 2006b) showed consistent differences in habitat use, foraging behaviour and diet. Nevertheless, my study shows that it is important to carry out ecological research to aid conservation when working with all cryptic bat species and not rely on the hypothesis that similar morphologies predict similar ecologies.

6.2. MANAGEMENT RECOMMENDATIONS

Clear evidence is lacking for population changes in either species, but given that both species have been subjected to the threats that have been implicated in the decline of many bat species (roost loss, timber treatment, agricultural intensification, habitat loss), it is likely that current population levels are lower than those perhaps 100 years ago (Vaughan 1996). *M. mystacinus* has a medium extinction probability based on their population density, home ranges, number of habitat types used and ability to live in matrix habitats (Bright 1993). No such information is available for *M. brandtii.* Land use in the UK has changed markedly in the past under the influence of human culture, and is likely to continue changing in the future. For example, between 1984 and 1990, the length of hedgerows decreased by 23% in the UK, but there was an increase in urban areas, woodlands and semi-natural land use types (Barr et al. 1993, Sinclair 1993). In south west England, between 1945 and 1990, rough grazing decreased in area by about 40% (Sinclair 1993). The total area covered by rivers and lakes is also decreasing slightly (Barr et al. 1993). With likely decreases in the bat populations, it is therefore important that appropriate habitat and prey management is carried out.

M. brandtii would benefit from the conservation of woodland. 20.2% of the insect taxa (Hemerobiidae, Ichneumonidae, Tipulidae, Bibionidae, Rhagionidae and Syrphidae) in the diet of *M. brandtii* are associated with woodland supporting the habitat use data. In woodlands, glades and road edges with shrubs and grass are good habitats for insects, and deciduous trees support more species of insect than conifers (Fry and Lonsdale 1991). Groups of trees should be left to mature, particularly in conifer plantations, where trees are normally cut as timber as soon as their growth rate slows down. Many woodland species of Diptera and Coleoptera are associated with dead or decaying wood, or with very mature trees (Sutherland and Hill 1995). The selection of coniferous woodland by *M. brandtii* in this study was surprising given the low insect diversity and restricted roosting possibilities in such

woodland. Coniferous woodland should be considered as a potentially important habitat around *M. brandtii* roosts.

M. mystacinus would benefit from the conservation of grassland. All types of grassland are used by the species, although grazed pasture seems to be preferred. 13.4% of the insect taxa (Carabiidae, Scarabiidae and Scathophagidae) in the diet of *M. mystacinus* are associated with grazed pasture supporting the habitat use data. Vegetation structure at the microhabitat level is also important for insect communities, and a reduction in grazing intensity for example, enhances insect diversity (Kruess and Tscharntke 2002). Insect densities are generally higher closer to vertical landscape elements than in open areas (Lewis 1969).

M. mystacinus and *M. brandtii* would probably benefit from the conservation of hedgerows. In grasslands, hedgerows and emergent trees are probably important microhabitats for Diptera and for foraging bats (Gaisler and Kolibáĉ 1992, Peng et al. 1992). Hedgerows may also be used as flight corridors for movement between habitats and as shelter from wind and predators (Limpens and Kapteyn 1991, Walsh 1995).

Habitat management should focus within 3.0 km of the roost, with a main focus within 1.0 km, given that the radiotracking showed that the *M. mystacinus* and *M. brandtii* in my study had a maximum foraging distance of 2.3 km and 3.2 km respectively, with an average of around 0.8 km. It will be beneficial if habitat fragmentation is kept low as none of the bats foraged in built up areas and only one *M. mystacinus* crossed the motorway (M49) at on of the radiotracking sites (Compton House, Bristol). DeJong (1994) and Johansson and DeJong (1996) also had similar conclusions in studies of *M. brandtii*. Habitat fragmentation can be expected to have two principal effects. Not only will bats have to travel greater distances to feeding areas, but the fragmentation of feeding habitats will increase the cost of commuting, with bats being unable to catch prey en-route to quality feeding areas.

Grazed pasture and woodland, the prime foraging habitats of *M. mystacinus* and *M. brandtii* have high densities and diversities of insects (Stebbings 1982). The conservation of Diptera and the habitats in which they breed is of particular importance as this is the main dietary component of both species. In order to conserve the prey, conserving the habitat may not be sufficient however, it is also important to keep in mind that bats may get exposed to pollutants through contaminated prey from pesticide use or use of the antihelminthic drug Ivermectin often used in cattle and sheep (Strong and James 1992). Refer to Parsons and Jones (2003) for

a discussion of the importance of conserving swarming sites in the conservation of *M. mystacinus* and *M. brandtii*. Further information on habitat management for the conservation of bats and invertebrates can be found in Entwistle et al. (2001) and Kirby (1992).

6.3. RECOMMENDATIONS FOR FUTURE WORK

The conservation of bats and their prey probably requires dedicated land management schemes. However, practical habitat management experience is rarely documented. Ideally, all studies on the effects of management on bat populations should take the form of controlled experiments and should be followed by detailed monitoring. For bats, foraging activity, numbers in roosts and breeding success should be measured in areas of rapid land use change, in order to evaluate the effects of land use management. Only with the results of such experiments can we hope to make accurate predictions about what lies ahead for populations of bats in the UK.

Further studies on habitat use, foraging behaviour and diet with a larger sample size of bats living both in allopatry and sympatry would yield some very interesting and useful information. The radiotracking and faecal sampling should preferably be carried out simultaneously. Studies should also be carried out in different geographic areas in the UK and on the continent. Also, habitat surveys around maternity colonies should be carried out within the maximum foraging distance of *M. mystacinus* and *M. brandtii* (2.3 and 3.2 km respectively) as this is less expensive and labour intensive than radiotracking so the samples sizes of bats and study sites could be larger. Studies on habitat use, foraging behaviour and diet should also be carried out using males and females at different stages of reproduction throughout the season e.g. there was significant variation in the habitat use of *Pipistrellus pipistrellus* during the season (Russ and Montgomery 2003). Additionally, the same individual bats should be studied in different years. Dietary studies should also be carried out at swarming sites in order to minimise temporal and geographical differences, in addition to the fact that bats feed in sympatry at these sites. Further identification features e.g. hair structure, wing venation, blood vessels in the uropatagium, position of auricles, nostril shape, slope of forehead, length of tibia, metacarpal and phalanges and dental characteristics (as described in section 2.4.1.6) should also be tested in order to find one, foolproof identification feature to distinguish between the two species.

Identification, habitat use, foraging behaviour and dietary studies are still needed in order to conserve the two species effectively. However, previous studies of *M. mystacinus* and *M. brandtii* are few or sometimes non existing. For example, there were no previous radiotracking studies investigating both species, previous studies have also never looked at morphological differences based on genetic identification. The existing studies on e.g. diet, habitat use and foraging behaviour are often difficult to compare or use methodology which is likely to have some inherent biases. *M. mystacinus* and *M. brandtii* were possibly the most understudied bat species in the UK and the "Action Plan for the Conservation of Bats in the United Kingdom" highlights that "further research is needed to establish ecological and conservation requirements of either species" (Hutson 1993). My study has therefore added valuable information on habitat use, foraging behaviour, diet and identification of *M. mystacinus* and *M. brandtii.*

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